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HEREDITARY VARIATION IN PLANT NUTRITION[1]

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Received January 6, 1939

INTRODUCTION

PLANT nutrition is here considered from the combined viewpoints of genetics and physiology. Present knowledge concerning plant nutrition as affected by heredity is scanty. STRINGFIELD and SALTER (1934) and LAMB and SALTER (1936, 1937) report a rotation of corn-oats-wheat conducted on four levels of fertility in which corn varieties and hybrids gave significant interactions with fertility levels two years out of five. Wheat showed a significant 'variety × level of fertility' interaction for the five years while oats did not. GREGORY and CROWTHER (1928) show five barley varieties differed significantly in their response to three levels of N, P, and K in all combinations.

Several established inbred strains of yellow dent corn and certain first generation crosses are reported by DETURK and his collaborators (1933) to differ in response to fertilizer treatments. Thirteen inbred lines of Early Yellow Dent corn and two hybrids gave differential growth when grown on clay and loam soils according to HOFFER (1926). Eight Hogue Yellow Dent inbreds, six White Prize inbreds and certain hybrids varied in their water economy as shown by KIESSELBACH (1926). None of these investigators demonstrated any correlation between the specific behavior of parent inbreds and hybrid combinations.

SMITH (1934) tested a number of corn inbred lines and certain hybrids on several levels of phosphorus and nitrogen. Marked differential responses were found between the inbred lines on low phosphorus levels and to a lesser degree on low nitrogen. Hybrids showed a strong tendency to approach the more efficient parent. Aqueous, sand, or soil-sand cultures all supported similar responses to the phosphorus series. Line responses remained the same whether measured as vegetative plant weight or ear weight. LYNESS (1936) also grew inbred strains and hybrids of corn on phosphorus and nitrogen treatments. His results agree well with those of SMITH'S. Both workers attribute efficiency in phosphorus utilization to a greater proportion of secondary to primary roots on efficient inbred lines and hybrids.

The larger section of this paper deals with the absorption and utilization of nitrogen ionic forms by corn (*Zea mays*) inbreds and hybrids. The remaining section is concerned with response to limited supplies of nitrogen, phosphorus, and potassium by tomato strains of *Lycopersicum esculentum* and *L. pimpinellifolium*.

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MATERIAL

Twenty-seven yellow dent inbreds, six sweet inbreds, one white dent inbred, one white flint inbred, one yellow flint variety and one white dent variety were tested. These represent inbred strains from Iowa, Illinois, Indiana and New Jersey. The two varieties were obtained from the Florida Agricultural Experiment Station. A brief description of the lines used most extensively is found in table 3, pp. 504–519 of the 1936 Yearbook of Agriculture. The sweet, white dent, and white flint inbred strains have been developed by the Genetics Department, Iowa State College, Ames, Iowa, as have most of the inbred dent corn lines.

Eighteen varieties or strains of *Lycopersicum esculentum* and five strains of *L. pimpinellifolium* were included. Two strains of the former and five of the latter species had been collected from the wild state in South America, chiefly Peru. Two varieties were imported from Italy and one from the Philippine Islands. Strains have all been inbred and purified as genetic stocks in the Genetics Laboratory at Iowa State College. All seeds used were gathered from greenhouse cultures to assure purity.

PROCEDURE

Uniform seed, dusted with Merko to reduce fungus growth, was germinated between paper toweling kept moist by strips of toweling extended into water below hardware cloth supports in granite ware germinating pans. These pans were placed in a germinating chamber for 36 to 48 hours with temperature between 35° and 40°C. At this time radicles were one-half to three-fourths of an inch in length. They were then placed on quarter inch mesh, paraffined hardware cloth supports which were cut to fit the pans so as to rest on the sides about half way up from the bottom. The water level was kept just below the hardware cloth. These pans were held in the dark germinating chamber another 24 hours and then placed in light. After three days the seedlings were large enough to be placed in corks of culture jars. By germinating twice the amount of seed necessary, very uniform seedlings were available for culture studies. The above procedure produced seedlings with very short mesocotyls, an important point in seedlings to be grown in water cultures.

The method of handling corn plants was as follows: Two uniform seedlings were fitted into a two-hole cork (60 mm diameter) with cotton which held the small plants in place and allowed them to expand by compressing the cotton. These corks with seedlings were then placed in quart Mason jars filled with tap water. Later the tap water was replaced by the solutions used. Solutions were changed at intervals of one to two weeks dependent on age of plants and greenhouse growing conditions. Tap water was added between solution changes when required.

Preliminary tests proved that even two brown paper bags slipped over

the jars did not check algal growth. For this reason all jars used were dipped in black screen paint and when dry, redipped in white paint. These containers checked algal growth successfully and did not heat excessively.

Plants were harvested after four to six weeks growth on solution. Green weight of tops was taken in all cases but is not included here. Dry weights of tops and roots were obtained after either thoroughly air drying the plants or oven drying them to a constant moisture percentage. Analysis of variance described by SNEDECOR (1934, 1937) was calculated for total dry weight of plants for all tests. All data represent two plants, that is, one culture jar.

Tomato seeds (for series I, II, and III) were sown in greenhouse flats of two-thirds sand and one-third soil compost mixture. When seedlings were in the two to four leaf stage they were dug, roots washed free of soil particles and plants placed in corks as done with corn seedlings. They were then put in jars of tap water. Five to seven days were allowed to elapse before nutrient solution replaced the water. Tap water partially depleted the nutrient reserve of seedlings and also favored the formation of new roots which were adapted to aqueous media.

Tomato seedlings for series IV were started in flats of sand watered with weak concentrations of a complete nutrient solution. This method proved more efficient in producing uniform seedlings. The nutrient reserve of plants could be controlled easier in sand cultures than in sand-soil mixtures.

The nutrient solutions were modifications of the buffered solution ZINZADZÉ (1933) recommends for constant pH, and the solution used by SMITH (1934). Tap water was used for all cultures making it necessary to add only iron to corn cultures and iron plus boron to tomato cultures in addition to major elements. Iron was added as ferric tartrate in small portions as plants needed it. Boron was added in the form of boric acid at 0.5 p.p.m.

Three modifications of the buffered solution were used in corn studies. These were designated as solutions A, B, and C according to nitrogen form used; NH_4NO_3, KNO_3, and NH_4HCO_3 respectively. The salts used and quantities of each were as follows:

Solution	Salt	Grams per liter of solution
A, B, and C	$MgSO_4$	0.500
A, B, and C	$Ca_3(PO_4)_2$	1.000 approximate
A, B, and C	KCl	0.500 "
A	NH_4NO_3	0.286
B	KNO_3	0.714
C	NH_4HCO_3	0.562

The two salts marked 'approximate' were added as the buffer which was freshly prepared for each solution change.

In each solution the nitrogen content was calculated to 100 p.p.m. regardless of the form supplied. In test VII one-half strength solutions were used, making nitrogen content 50 p.p.m.

Also three modifications of SMITH'S solution were utilized in some of the corn tests. They were designated solutions D, E, and F according to the nitrogen salt used. Nitrogen was again calculated to be approximately 100 p.p.m. Solutions were as follows:

Solution	Salt	Grams per liter of solution
D, E, and F	KCl	0.576
D, E, and F	$MgSO_4$	0.864
D, E, and F	KH_2PO_4	0.132
D	$(NH_4)_2SO_4$	0.144
	$Ca(NO_3)_2$	0.400
E	$Ca(NO_3)_2$	0.877
F	$(NH_4)_2SO_4$	0.708

Solutions for the tomato series were essentially the same as SMITH (1934) used for maize. In making up partially deficient nitrogen solution, $CaCl_2$ was used in place of $CaSO_4$. Minus potassium solution was prepared by substituting $MgCl_2$ for KCl and $Ca(H_2PO_4)_2$ for KH_2PO_4.

During a preliminary test hydrogen-ion content of solutions was determined electrometrically. Initial pH varied from 7.2 for solutions A and B to 7.4 for solution C. The tap water used in these solutions had a pH of 7.6. After plants had been in solutions seven days, solutions A and B had a pH of 7.3 and solution C still had a pH of 7.4. It may be noted that all solutions were slightly alkaline. This checks closely with SMITH'S work using the same source of tap water. Because of the large amount of tap water used the comparatively small amount of salts had little influence on pH. It was not considered necessary for this investigation to follow the pH of solutions more closely since such small differences existed between solutions.

Slight alkaline reaction should favor the absorption and utilization of NH_4-N and at the same time not greatly influence the absorption and utilization of NO_3-N. LOO (1931) found corn seedlings absorbed more ammonia as the acidity of the solution decreased. Nitrate nitrogen was absorbed most rapidly from acid media but was less influenced by reaction of solution than was ammonia.

RESULTS

Corn tests[2]

Seven inbred strains of corn were cultured on solutions A, B, C, and D from February 6 to March 12, 1936. The test was made in duplicate

[2] Eight corn tests were made. Those omitted here along with some of the data for tests included may be found in the author's doctoral thesis, #470, Iowa State College Library, Ames, Iowa.

randomized as one unit. Fresh solution was supplied three times. Mean total dry weight data and their analysis of variance summary are presented in tables 1 and 2.

TABLE 1

Mean total dry weight in grams of plants and coefficient of variability for inbred strains. Test I (corn).

INBRED LINES	SOLUTION				C.V.
	A	B	C	D	
Mc	9.5	9.6	6.5	7.4	18.6
La	9.1	8.9	8.5	8.3	4.1
PR	7.5	6.8	8.4	8.5	10.3
K187	8.7	8.8	7.8	8.6	5.4
WCR	10.1	10.2	10.3	9.3	4.6
Ldg(Osf)	11.1	11.0	8.1	10.6	13.9
Idt	7.7	8.1	5.8	8.8	16.9
Mean	9.10	9.06	7.91	8.79	10.5

TABLE 2

Analysis of variance based on total dry weight of plants. Test I (corn).

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between strains	6	8.13**
Between solutions	3	4.38**
Interaction	18	1.71*
strains×B-C	6	2.97**
remainder	12	1.08
Error	28	0.69
Total	55	

** Highly significant. (P less than .01.)

* Significant. (P less than .05.) In all succeeding tables the same custom of using asterisks in the Mean Square column will be used.

Interaction mean square lies between the five percent and one percent levels as measured by its F value and the values given in table 10.2 of SNEDECOR'S "Statistical Methods" (1937). When this source of variation is broken down further the six degrees of freedom attributable to 'strains × solution B-C' are found to contribute a portion of the variance greater than their proportional part. Thus their mean square is highly significant. By comparing strains with solutions B (NO_3) and C (NH_4) a direct measure of the differential response by strains to nitrate only and to ammonium only is obtained. From this it may be stated that these seven inbreds differed in their response to nitrate versus ammonium nitrogen form under the conditions of this test.

Differential strain responses to the two forms of nitrogen are shown in figure 1. Inbreds Ldg(Osf), Mc, and Idt show proportionally less growth on ammonium-nitrogen than do the other four strains.

Top-root ratios of plants were determined since a noticeable discoloration, varying in intensity between strains, was observed on roots grown in solution C. Table 3 presents the mean top-root ratios for three solutions.

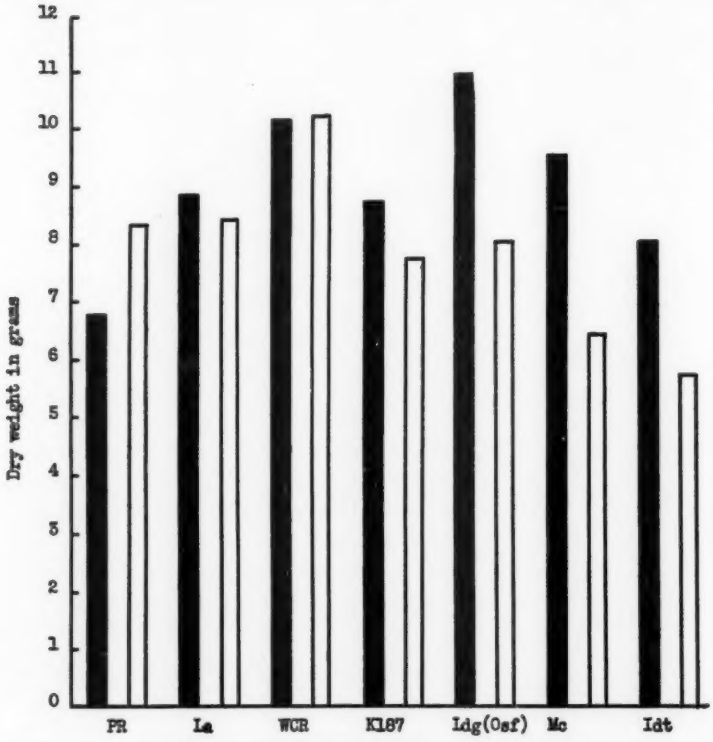

FIGURE 1.—Mean total dry weight for solutions B(NO_3-N, solid bars) and C(NH_4-N). Test I.

Differences between means of strains in table 3 are interesting. La and K187 have narrow ratios (2.80 and 2.58) which means that these have proportionally heavier roots than other strains. These differences between strains are highly significant statistically while no significance can be assigned to the variance between solutions or the variance due to the interaction of strains and solutions. Therefore, any toxicity which resulted from solution C must have affected top and root alike so far as dry weight

TABLE 3

Average top-root ratios based on dry weight of plants. Test I (corn).

STRAIN (INBRED)	SOLUTION			MEAN
	A	B	C	
Mc	3.37	3.25	3.28	3.30
La	2.72	3.00	2.68	2.80
PR	3.32	2.99	2.74	3.01
K187	2.40	2.68	2.66	2.58
WCR	3.38	3.42	3.12	3.30
Ldg(Osf)	3.30	3.30	3.32	3.31
Idt	3.22	2.91	2.87	3.00
Mean	3.10	3.08	2.95	3.07

is concerned. Since the interaction variance is very low no important relationship between top-root ratios and response to nitrogen form could have been present.

Percent dry matter in tops was also determined because some plants grown on ammonium nitrogen appeared to be more succulent than plants grown on nitrate nitrogen solution. The analysis of variance summary based on these percentages is given in table 4. Plants grown on ammonium

TABLE 4

Analysis of variance based on percent dry matter of tops. Test I (corn).

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between strains	6	3.45**
Between solutions	2	1.17**
Interaction	12	0.29**
Error	21	0.09
Total	41	

** P less than .01.

nitrogen produced slightly less dry matter than plants grown on other nitrogen forms. The means for solutions exhibit a range of more than twice the standard error (±0.11 percent). Strains differed significantly in the amount of dry matter they produced. Ldg(Osf), PR, and La have mean percentages of dry matter of tops more than twice the standard error for strains (±0.17) greater than K187, Idt, WCR, and Mc.

The interaction of strains and solutions (table 4) is highly significant, that is, strains did not all produce the same relative percent of dry matter on the different solutions. Strains, such as PR and WCR, which produced total dry weight on ammonium solution equal or nearly so to that pro-

duced on nitrate solution also had higher relative percentages of dry matter on ammonium solution than did other strains. This differential strain response in percent dry matter would seem to indicate a difference in the utilization of ammonium nitrogen by different inbred strains of corn.

Test II was grown immediately following the harvest of test I. Twenty inbred strains and one variety were grown on three solutions. The same plan was used as in test I. Table 5 contains the data of this test.

TABLE 5

Mean total dry weight in grams and coefficient of variability of strains. Test II (corn).

STRAIN	SOLUTION			C.V.
	A	B	C	
Mc	9.6	8.4	4.8	32.9
La	10.0	9.3	10.3	5.2
PR	9.7	7.5	7.1	17.3
K187	8.5	7.6	6.7	11.8
WCR	9.0	8.6	7.4	10.0
Ldg(Osf)	11.3	10.5	10.9	3.7
Ldg(Blx)	8.5	7.8	4.5	30.8
Ldg	7.1	7.1	5.4	15.0
Bls	8.7	6.7	7.6	13.1
Ose	10.3	10.0	8.1	12.6
Km2	10.6	10.9	8.8	11.2
PR-M	8.9	7.1	7.3	12.7
Sto	7.4	6.5	4.2	27.4
WF9	9.6	8.8	8.2	7.9
Hy	7.6	8.2	4.5	29.3
R4	6.9	7.3	4.7	22.2
LP159	8.3	7.9	4.1	34.2
TR	7.9	8.3	5.5	25.0
A-47	10.0	9.4	6.9	18.8
Tuxpan	10.4	9.1	11.6	12.1
G.B. 797	8.0	6.3	6.0	15.9
Mean	8.97	8.25	6.88	18.02

The coefficient of variability of strains varied greatly in this test. Mc has a large coefficient of variability while La, K187, and WCR have comparatively low values. It may be observed that these coefficient of variability values are large or small mainly because the strain did or did not respond favorably on solution C.

The analysis of variance shows that the variance between strains and between solutions contributes highly significant portions. The interaction, strains with solutions, was likewise highly significant. Inbreds Mc, La, PR, K187, and WCR were consistent in their response in tests I and II. No explanation can be given for the inconsistent results from Ldg(Osf).

TABLE 6

Average top-root ratios based on dry weight of plants. Test II (corn).

STRAIN	SOLUTION			MEAN
	A	B	C	
Mc	2.67	2.39	2.89	2.65
La	2.51	2.35	2.05	2.30
PR	3.17	2.93	2.60	2.90
K187	2.10	1.83	2.01	1.98
WCR	2.87	2.49	2.51	2.62
Ldg(Osf)	3.88	3.04	3.07	3.33
Hy	2.33	2.05	2.44	2.27
R4	3.08	2.98	3.19	3.08
Mean	2.83	2.51	2.60	

Top-root ratios were determined for eight strains (table 6). Inbreds K187, La, Mc, WCR, and Ldg(Osf) have the same ranking of means of strains as they had in table 3. The difference between strains is again highly significant. The difference between solutions is just statistically significant and no significant relationship between strains and solutions was found.

Percent dry matter of tops for eight strains was calculated (table 7). As in the previous test the mean percent dry matter for solution C is lower than for either solutions A or B (standard error for solutions $\pm .15$ percent). Also strains have statistically significant differences between their mean percent dry matter (standard error for strains $\pm .24$ percent). Strains Ldg(Osf), PR, and La have higher percentages of dry matter than K187, WCR, and Mc again agreeing with data in test I. Differential response to

TABLE 7

Mean percent dry matter of tops for eight strains grown in Test II (corn).

STRAIN	SOLUTION			MEAN
	A	B	C	
Mc	9.7	9.6	8.5	9.27
La	12.4	11.7	12.2	12.10
PR	12.4	12.8	10.6	11.93
K187	10.4	10.3	9.5	10.07
WCR	9.6	9.7	8.4	9.23
Ldg(Osf)	14.0	13.7	12.9	13.53
Hy	11.1	10.9	9.4	10.47
R4	11.0	11.2	9.3	10.50
Mean	11.33	11.24	10.10	

treatment by strains was less important in this test than in the previous one. The interaction mean square is just at the five percent level. Only one strain, La, had as high a percent dry matter on solution C as on other solutions.

TABLE 8

Mean total dry weight of plants in grams. Test V (corn).

STRAIN	ZINZADZÉ'S SOLUTION			SMITH'S SOLUTION		
	A	B	C	D	E	F
Hy	7.1	6.4	5.1	8.7	7.8	5.5
R4	7.5	7.4	4.4	6.5	6.0	6.1
Mc	6.7	5.5	4.1	6.9	5.0	6.1
PR	5.9	6.4	5.6	7.1	6.1	6.4
La	8.4	8.5	6.1*	8.8	7.9	6.7
WCR	9.1	7.5	5.4	9.7	8.9	6.2
Mean	7.45	6.95	5.12	7.95	6.95	6.12

* Data were missing because of root rot. Yields filled in by means of missing plot technique given by YATES (1933).

TABLE 9

Analysis of variance based on total dry weight. Test V (corn).

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between blocks	2	0.25
Between solutions†	1	7.02**
Between strains	5	12.80**
Between N-forms‡	2	38.93**
Interactions:		
sol.×strains	5	1.21
sol.×N-forms	2	2.69
strains×N-forms	10	2.19*
sol.×str.×N-forms	10	1.33
Error	67§	0.98
Total	107	

† "Solutions" refers to ZINZADZÉ's and SMITH's type.

‡ "N-forms" refers to ionic forms separately and together.

§ One D/F is deducted for each datum supplied by missing plot technique.

** P less than .01.

* P less than .05.

In order to measure response to nitrogen forms when they are associated with different combinations of other nutrient elements test V was designed. The same buffered solutions (A, B, and C) were used and three modifications (D, E, and F) of SMITH's solution were added. Each solution was triplicated with each of six inbreds; Hy, R4, and Mc shown by earlier

tests to be inefficient in ammonium utilization and PR, La, and WCR shown to be efficient. Randomized block design was first made use of in this test and continued in all later tests. Plants were put into solution March 20 and harvested April 20, 1937 (tables 8 and 9).

The interaction of strains by N-forms is the only one which may be said to have any degree of significance. It also happens to be the one which is of most interest. From the small mean square attributable to the interaction, solutions × strains, it is evident that these six strains responded similarly to the two solution types.

Inbreds PR, La, Mc, Hy, and R4 were chosen for hybridization because they represented extreme types in ammonium utilization. Hand pollinated single cross hybrids with endosperms removed were used in both hybrid tests. Endosperms were removed when seedlings were placed in cork supports. In a small side experiment grown in connection with test V it was observed that removing endosperms checked bacterial growth in cultures. No putrefying bacteria were observed in any culture in which endosperms were removed.

Test VII was grown from April 27 to June 2, 1937. Two strengths of each solution were included; 'full' strength was same as in earlier tests and 'half' strength was just one-half concentration for all elements. Results are given in table 10 and statistical data in table 11.

TABLE 10

Mean total dry weight in grams of hybrid plants. Test VII (corn).

HYBRID	SOLUTION					
	A		B		C	
	FULL	HALF	FULL	HALF	FULL	HALF
Mc×Hy	14.4	10.8	14.9	9.9	11.9	9.9
R4×Mc	13.2	12.3	13.3	10.4	12.4	10.1
PR×Hy	13.5	11.3	14.3	11.0	11.6	9.7
PR×Mc	15.4	13.1	17.2	12.2	14.6	10.7
La ×Hy	15.4	12.0	14.3	10.5	12.6	10.7
R4×Hy	10.2	9.6	11.6	9.9	6.8	7.6
Hy×R4	13.5	10.9	11.9	10.6	7.8	8.9
Mean	13.66	11.43	13.93	10.64	11.10	9.66

Hybrids responded differentially to nitrogen forms and strengths of solutions. The interaction 'solutions (N-forms) × hybrids' is highly significant. 'Hybrids × strengths' is also highly significant. An examination of table 10 shows all hybrids to have made more growth on 'full' than on 'half' strength solutions except the reciprocals R4×Hy and Hy×R4 on solution C.

TABLE 11

Analysis of variance based on total dry weight of hybrids. Test VII (corn).

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between blocks	2	13.20**
Between solutions	2	58.84**
Between strengths	1	169.39**
Between hybrids	6	37.84**
Interactions:		
sol.×hybrids	12	2.56**
sol.×strengths	2	9.12**
hybrids×str.	6	7.02**
sol.×hyb.×str.	12	1.42
Error	82	1.03
Total	125	

** P less than .01.

Fifteen hybrid combinations of the five inbreds Mc, PR, La, Hy, and R4 were grown from September 4 to October 16, 1937. Only solutions A, B, and C were used with five replications. Statistical data for this test are found in table 12.

TABLE 12

Analysis of variance based on total dry weight of hybrids. Test VIII (corn).

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between blocks	4	1.11
Between solutions	2	325.44**
Between strains	14	26.22**
Interaction	28	1.78
5−7×B-C†	1	8.32**
5+7−(2)8×B-C	1	0.86
5+7+8−(3)13×B-C	1	0.56
5+7+8+13−(4)2×B-C	1	11.25**
5+7+8+13+2−(5)11×B-C	1	3.85
Remainder	23	1.07
Error	176	1.84
Total	224	

† Numbers 5, 7, 8, 13, 2, and 11 refer to hybrids Mc×Hy, R4×Mc, R4×Hy, Hy×R4, PR×La, and La×PR respectively.

** P less than .01.

Total interaction in this test was not significant but this should not be taken to mean that hybrid strains all responded similarly to the treatments. When the majority of strains react similarly as they do here the

variance brought into an analysis by a few strains is overshadowed. If the interaction is broken up into smaller units certain of these comparisons are significant. Two of the five individual comparisons chosen from the 28 interaction comparisons were significant.

The reactions of all hybrid strains grown are given in table 13 as mean differences. The mean differences, (A+B)/2C, is a statistical treatment used to isolate strain response by solutions.

TABLE 13

Mean differences, (A+B)/2C, of total dry weight of all hybrid strains in grams (corn).

HYBRID STRAIN	MEAN DIFFERENCE		AVE. M.D. FOR GROUP	
	TEST VII	TEST VIII	TEST VII	TEST VIII
Inefficient×inefficient*				
Mc×Hy	1.6	3.7		
R4×Mc	1.1	5.0	2.30	4.20
R4×Hy	3.1	4.6		
Hy×R4	3.4	3.5		
Inefficient×efficient				
R4×La		3.0		
R4×PR		3.1		3.13
Hy×La		3.2		
Hy×PR		3.2		
Efficient×inefficient				
PR×Mc	1.8	2.4		
PR×R4		3.9		
PR×Hy	1.8	3.2	1.67	3.78
La×R4		4.9		
La×Hy	1.4	4.5		
Efficient×efficient				
La×PR		3.1		2.85
PR×La		2.6		

* Grouped according to the response of inbred parent strains. Maternal parent always written first.

Examination of the average mean differences for groups in table 13 shows an association between hybrid responses and the response of their respective parental strains. A large mean difference represents inefficient utilization of ammonium nitrogen by that hybrid. The large average mean difference for the group 'inefficient×inefficient' (4.20 grams, test VIII) illustrates the inefficiency in ammonium utilization by hybrids of inefficient parent lines. At the same time, the average mean difference for group 'efficient×efficient' (2.85 grams, test VIII) is the smallest group average and demonstrates the relative efficiency of hybrids of efficient parent strains.

Tomato studies

Three series of tomatoes were grown on four solution treatments; full nutrient as used by SMITH, low N, low P, and minus K. Each series was run in duplicate and completely randomized. Air dry weight of plants was obtained. The series were cultured during the periods June 1 to June 27; August 26 to October 1; and October 11 to November 9, 1935 respectively. Solutions low N and low P in series I had one-tenth the amount of the respective element present in full solution. In series II these two solutions were made one-fifth the concentration of full solution for the respective elements. Low N was one-fifth and low P was one-tenth the concentration of full solution in series III.

TABLE 14

Mean differences in grams of total dry weight on low N, low P, and minus K compared with full solution of series I; strains grouped according to fruit size (tomato).

STRAIN	FULL-LOW N	FULL-LOW P	FULL-MINUS K
Small fruit:			
Red Currant	4.2	4.2	2.3
Peru	3.5	3.6	1.9
Lima	3.5	3.5	2.3
Lima II	3.2	3.3	1.6
S. racemigerum†	3.2	3.4	1.8
Mean	3.52	3.60	1.98
Medium fruit:			
Mulua	2.9	3.0	3.1
Guatemala City	2.6	2.9	2.1
Yellow Cherry	3.4	3.9	2.8
Red Cherry	2.9	3.0	1.9
Burbank's Preserving	1.5	2.1	1.3
Mean	2.66	2.98	2.24
Large fruit:			
Philippine	2.7	3.1	1.9
Pizzitelo	2.3	2.6	2.1
Fraschelle	2.6	2.8	2.0
Model Potato Leaf	1.3	1.9	1.4
Yellow Peach	1.8	2.0	1.4
L. Gold Ball	2.3	2.8	2.3
Pritchard	2.4	2.6	1.4
Bonny Best	2.2	2.3	1.5
Livingston's Globe	2.5	3.0	2.2
Sutton's Best of All	2.3	2.8	2.0
Mean	2.24	2.59	1.82

† This is an intermediate form which is here grouped with *L. pimpinellifolium.*

In table 14 mean differences for series I are given with strains grouped according to size of fruit. The small-fruited strains are of the 'wild' species (*L. pimpinellifolium*); medium size strains are small 'cherry type' fruit of the domestic species; and all other strains were large fruited. Table 14 shows an interesting comparison between fruit size and dry weight response to low levels of mineral elements. It appears from this table that large fruited strains were in general more efficient on low N and low P than were small fruited strains. However, it should be again mentioned that all strains in 'small fruit' class are of a different species. Were it not that the 'medium fruit' class is intermediate these relationships could be explained as species differences. It is quite likely that some of the differences should be attributed to species. No relationship is evident between fruit size and dry weight response to minus K.

TABLE 15

Analysis of variance based on total dry weight. Series I, II, and III (tomato).

SOURCE OF VARIATION	DEGREES OF FREEDOM			MEAN SQUARE		
	I	II	III	I	II	III
Between strains	19	7	10	0.79**	5.10**	1.40**
Between solutions	3	3	3	68.84**	39.51**	20.15**
Interaction	57	21	30	0.29**	0.85**	0.43**
strains×N†	19	7	10	0.50**	1.22**	0.66**
strains×P	19	7	10	0.37**	1.19**	0.38*
strains×K	19	7	10	0.22*	0.71**	0.36*
Error	77‡	31	42	0.13	0.12	0.14
Total	159	63	87			

† These are selected groups of comparisons not merely a dividing of the 57 D.F. for interaction into three equal parts. Their sums of squares do not add to equal the sum of squares for interaction because a portion of the variance is repeated in each group of 19 D.F.

‡ One D.F. is deducted for each datum supplied by missing plot technique.

** P less than .01.

* P less than .05.

Because of the similarity of the three series the statistical data are pooled in table 15. The main interactions of strains×solutions are highly significant in each series. Further, when these interactions are broken down to strains by each element separately all three elements are found to contribute a significant portion in each series. Such consistent behavior by tomato strains leaves no doubt of their inherent differences. These strains differ not only in their ability to utilize limited supplies of nutrients as shown by the general interactions but further differ in their ability to use a limited supply of any one of the three elements used.

Five strains were chosen for crossing and further study on the potassium solutions. These are: Lima II, *S. racemigerum*, and 2n ex Haploid as efficient potassium types and Mulua and Yellow Cherry as inefficient potassium types. Table 16 gives the response of each strain used later in hybrids on minus K solution in percentage based on its response on full solution as 100 percent.

TABLE 16

Percentage response on minus K solution based on each strain's response on full solution as 100 (tomato).

STRAIN	SERIES			AVERAGE
	I	II	III	
Lima II	69	79	64	70.7
S. racemigerum	65	62	70	65.7
2n ex Haploid	—	64	73	68.5
Mulua	31	42	—	35.5
Yellow Cherry	48	52	48	49.3

Series IV was grown two years after the three series already reported. Plants were put into solution October 21 and harvested December 3, 1937. Five strains chosen for their potassium response and all but one of their possible hybrid combinations were grown. Because of the high Calcium content of tap water it was difficult to hold iron in solution. Therefore, one-half of $Ca(NO_3)_2$ was replaced by the equivalent NO_3 as $NaNO_3$ in SMITH'S solution which helped greatly in maintaining iron in the plants. Five replications of each parent strain and four replications of each hybrid were included. However, root rot (a browning and sloughing off of roots) made it necessary to discard heavily at harvest. Data for this series are presented in table 17 and analysis of variance in table 18. The method of analysis used was that given by SNEDECOR (1934) for disproportionate class frequencies.

The highly significant mean square found for interaction indicates that these strains and their hybrids responded differentially to the two levels of potassium. When mean total dry weight for all parent strains was compared with the corresponding mean for all hybrids the latter were found to have yielded an average of 0.39 grams more per culture on full solutions and 0.84 grams more per culture on minus K solution than had the parents. This is an increase of 5.3 percent over parent mean for full solution and 21.2 percent increase over parent mean for minus K solution by the hybrids. In other words, this group of hybrids made more growth than their parents but this hybrid vigor was four times as pronounced on the deficient level as it was on the higher level of potassium. SMITH (1934) reported a similar increase of maize hybrids on moderately low levels of phosphorus.

TABLE 17

Mean total dry weight in grams, mean differences of full—minus K solutions, and leaf grade. Series IV (tomato).

STRAIN OR HYBRID	SOLUTION		MEAN DIFF.	LEAF GRADE*
	FULL	MINUS K		
Lima II (L)	6.6	4.3	2.3	II
S. racemigerum (S)	8.1	4.4	3.7	II
2n ex Haploid (H)	6.9	4.0	2.9	I
Mulua (M)	7.3	2.6	4.7	V
Yellow Cherry (Y)	8.3	4.0	4.3	IV
Mean	7.44	3.86		
M×Y†	8.3	3.8	4.5	IV
Y×M	7.7	3.3	4.4	IV
M×L	8.8	4.6	4.2	III
Y×L	7.6	4.1	3.5	II
M×S	7.9	5.0	2.9	IV
Y×S	8.6	6.4	2.2	IV
M×H	9.8	4.0	5.8	III
Y×H	6.9	4.0	2.9	III
L×M	7.1	5.0	2.1	III
L×Y	8.8	5.8	3.0	III
S×M	6.5	4.0	2.5	III
H×M	8.9	3.7	5.2	III
H×Y	7.1	5.1	2.0	IV
L×S	7.1	4.7	2.4	II
S×L	7.0	5.0	2.0	II
L×H	6.5	4.3	2.2	I
H×L	7.1	5.7	1.4	III
S×H	8.1	5.2	2.9	II
H×S	8.3	5.8	2.5	II
Mean	7.79	4.70		

* Leaf grades were obtained by observation at time of harvest, after 42 days on minus K solution. Grades are: I—just noticeable paling of leaf margins and occasional necrotic area on oldest leaves; II—noticeable yellowing of leaf margins, few necrotic areas; III—yellow leaf margins, few necrotic areas; IV—striking yellowing of leaf margins, necrotic areas common; and V—yellow leaf margins, necrotic areas very numerous, and old leaves dead.

† Letters represent parent strains, see brackets above.

TABLE 18

Analysis of variance based on total dry weight. Series IV (tomato).

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between strains	23	1.17**
Between solutions	1	113.47**
Interaction	23	0.67**
Error	97	0.13
Total	144	

** P less than .01.

Mean differences (full—minus K) for hybrids follow the same trend as the corn hybrids. Grouped according to their parent strains' response they have group averages as follows: inefficient×inefficient, 4.45 grams; inefficient×efficient, 3.58 grams; efficient×inefficient, 2.96 grams; and efficient ×efficient, 2.23 grams.

Leaf grades of potassium starvation symptoms were taken on this series and are recorded in table 17. Parent strains showed very different symptoms when grown on minus K solution. The dwarf 2n ex Haploid showed no symptoms other than reduced growth after five weeks. On the other hand, Mulua showed numerous small necrotic areas on older leaves after two weeks. By harvest time (six weeks) the lower four or five leaves were dead on all Mulua plants grown on minus K solution. Other strains and, in general, hybrids were intermediate in the degree of symptoms shown. Hybrids M×Y and Y×M graded low as did both parents. Hybrids L×S, S×L, L×H, H×L, S×H, and H×S graded fairly high thereby resembling their parents. Other hybrids were generally intermediate in their grade so that no conclusion could be made concerning dominance.

Observation of mean differences of reciprocal crosses in table 17 indicates that there might be some maternal influence acting in these hybrids causing them to respond to potassium deficiency somewhat in the same manner as their maternal parent. A direct comparison of these reciprocals is given in table 19. In each reciprocal pair the hybrid having an inefficient

TABLE 19

Comparison of mean differences of reciprocal tomato hybrids.

INEFF.†×EFF.		EFF.×INEFF.		DIFFERENCE OF RECIPROCALS
HYBRID	M.D.	HYBRID	M.D.	
M×L	4.2	L×M	2.1	2.1
Y×L	3.5	L×Y	3.0	0.5
M×S	2.9	S×M	2.5	0.4
M×H	5.8	H×M	5.2	0.6
Y×H	2.9	H×Y	2.0	0.9
			Mean	0.9±0.31

† Maternal parent reaction always given first.

maternal parent gave the greater mean difference. The mean of 0.9 grams is significant since its standard error is ±0.31 grams. Although the number of comparisons is small it may well be that some maternal influence is involved. SCHLÖSSER (1935) has noted maternal influence for tomato characteristics which carried over into the F_2 generation. Differences in osmotic value of cytoplasm was found for parent stocks and reciprocal hybrids.

Each cross closely resembled its maternal parent. If such differences as osmotic value exist between strains used in the present investigation they might well account for the tendency of hybrids to resemble their maternal parent in low K response.

Chemical analyses for total potassium were made on the parent strains. The chemical method used was that given by the Association of Official Agricultural Chemists (1935). Plants were taken after dry weight was obtained on them and the entire two plants in each sample ashed together. Averages of two samples from each treatment are given in table 20. This table shows a gradient in the amount of total K present in plants grown on

TABLE 20

Chemical analyses for total K of parent strains in grams. Series IV.

STRAIN	SOLUTION	AVERAGE K PER CULTURE	AVERAGE DRY WT. PER CULTURE	AVERAGE K ABSORBED PER GM DRY WT.
Mulua	full	0.2283	5.79	0.0393
Yel. Cherry	full	0.3651	8.60	0.0425
Lima II	full	0.2907	6.27	0.0464
S. racem.	full	0.3073	6.59	0.0507
2n ex Haploid	full	0.3231	7.37	0.0439
Mulua	minus K	0.0263	2.81	0.0094
Yel. Cherry	minus K	0.0252	4.07	0.0062
Lima II	minus K	0.0326	4.24	0.0077
S. racem.	minus K	0.0299	4.43	0.0068
2n ex Haploid	minus K	0.0370	3.96	0.0094

minus K solution. Inefficient varieties (based on dry weight of plants) had less K present than efficient varieties. Of those plants grown on full solution the same holds true except for Yellow Cherry. This one variety not only made the largest growth but had the most K present of any variety grown on full solution. Too much variation is present in the data based on K absorbed per gram of dry weight to tell what really has taken place. A slight tendency for efficient varieties to have absorbed more K per unit of plant is present in plants grown on full solution.

DISCUSSION

Plant species are frequently assumed to be relatively homogeneous physiological populations. Physiological differences between species are well recognized but seldom are such differences considered within a species. The present investigation has brought to light such inherent physiological differences within species. Corn and tomatoes have shown highly significant differential strain behavior when tested for nutritional responses. That is, when strains of a species were grown on several nutritional solu-

tions their rank (measured as total dry weight of plants) was altered from one nutritional treatment to another. This change in rank by strains from one treatment to another was consistent between tests made in different seasons.

Corn inbred strains grown in aqueous mineral cultures having nitrogen either as anion (nitrate nitrogen) or as cation (ammonium nitrogen) consistently responded differentially to the two nitrogen forms. Inbreeding has thus isolated distinct physiological types, each inbred strain being an individual type. Most inbred strains produce more dry weight if supplied anion nitrogen. However, a minority of strains were capable of using the cation equally as well as the anion form provided the former was not too concentrated.

Tomato strains (and varieties) showed consistent, statistically significant, differential strain growth on high and low levels of nitrogen, phosphorus, and potassium solutions. Some strains because of their genetic constitution were capable of making more relative growth on a limited supply of an essential element than were others.

It should be noted that uncontrolled or environmental variation was minimized in this study by using randomized replicated tests and analysis of variance for statistical treatment. Hence the differences found are those resulting from the genetic make-up of the test biotypes and the nutrient treatments. The differential strain responses mentioned above are the interactions of the genetic complex of strains compared with the nutritional treatments. Since the treatments were the same for each strain the significant 'interaction mean square' signifies genetic differences in physiology between strains.

Responses to nutritional tests made by corn and tomato hybrids of varying parentage illustrate the heritability of the nutritional complexes. LYNESS (1936) and SMITH (1934) reported inheritance of factors for efficient utilization of limited phosphorus and nitrogen by corn inbreds and their hybrids. Both investigators concluded dominance of factors for efficient utilization to be practically complete. In our study tomato hybrids grown on limited potassium gave intermediate responses not equal to their more efficient parent. Corn hybrids also responded intermediately to nitrogen forms when one parent was more efficient in ammonium utilization than the other parental inbred. The intermediate response to all F_1 hybrids having only one efficient parental strain indicates either partial dominance or a mode of inheritance with dominance divided between factors for efficiency and those for inefficiency. The latter interpretation seems preferable since several different strains were involved in the hybrids which all showed similar intermediate responses. If only a few genes were acting it would seem very unlikely that the hybrids of all strains and their

response on two sets of nutritional treatments should possess so nearly the same degree of dominance.

Corn inbred strains grown on solutions supplied with different nitrogen forms varied in dry matter produced per unit of green weight of tops (percent dry matter). In general, plants grown on ammonium nitrogen had lower percentages of dry matter than plants grown on nitrate nitrogen solutions. However, this was not true for all strains. Those strains which made relatively good growth on ammonium nitrogen also had comparatively higher percentages of dry matter than did strains which made relatively poor growth on the cation form. Thus, those strains which made proportionally better growth on cation nitrogen also had higher percent dry matter of tops. In the absence of chemical analyses it seems reasonable to interpret this association between high total dry weight of plants and percent dry matter for efficient ammonium-utilizing strains as indicative of metabolism of ammonium nitrogen more nearly like that of nitrate nitrogen. Empirical observations of plants at harvest agree with this deduction. Ammonium-grown plants of inefficient ammonium-utilizing strains were more succulent (that is, less differentiated) than nitrate-grown plants. However, those efficient ammonium-utilizing strains appeared as differentiated on ammonium as on nitrate solution.

On the other hand, the results from two strengths of solution indicate some toxicity of ammonium nitrogen when supplied at 100 p.p.m. of nitrogen. HOENER and DETURK (1938) report severe toxic symptoms on corn seedlings started in solution at 100 p.p.m. of ammonium nitrogen. STEWART et al (1925) found pineapples made better growth on 40 p.p.m. than on 160 p.p.m. of ammonium nitrogen. The higher concentration was extremely toxic as shown by poor root systems and poor tops. Since most of the corn in this investigation was grown on solutions supplied with 100 p.p.m. of nitrogen and since noticeable discoloration of some root systems grown in ammonium solutions was observed, it seems logical to attribute at least part of the observed differential response to the degree of ammonium tolerance possessed by the strains involved.

ARNON (1937) found that all treatments which favored oxidation in the solution benefited plants grown in NH_4-N (ammonium nitrogen). Aeration, copper, manganese, molybdenum, nickel and chromium were the treatments used. Since these treatments were more effective in NH_4-N than in NO_3-N (nitrate nitrogen) solutions, ARNON believes it is unsound to interpret responses noted in terms of nitrogen source alone. This is very likely true but in the present work tap water was used in all solutions. It scarcely seems possible that all these minor elements would be absent from tap water beyond the trace required. Further, ARNON used a level of NH_4-N which was definitely toxic whereas in the present work a level of

NH_4-N was chosen just at the threshold between non-toxicity and toxicity. This is illustrated when certain strains did not show any evidence of injury while others did. Therefore, it seems reasonable to think of the differential strain responses reported here as being measures of ammonium compared to nitrate forms of nitrogen as corn nutrients.

Corn inbred strains differed widely in coefficients of variability, which measured variation in response to nitrogen forms by strains. Inbred strains which utilized both forms equally well have a low coefficient of variability. Other strains have a high coefficient of variability which indicates failure to utilize the nutrient treatments equally. Ammonium nitrogen was generally the poorest nitrogen source and a strain's response on this form accounts for the size of its coefficient of variability. The ranges in coefficient of variability were 4.1 to 18.6 percent, 3.7 to 34.2 percent, and 12.3 to 27.4 percent for three tests. The low values indicate uniformity in nutritional responses equal to that found for morphological characters. KIESSELBACH and WEIHING (1933) found the following coefficients of variability in a corn variety: height of plants 10.4 percent, ear height 15.6 percent, stalk diameter 10.7 percent, length of interval from planting to silking 5.3 percent, and grain yield per plant 70.8 percent. LINDSTROM and KOOS (1931) report variations in a genetically pure line of tomatoes, a diploid regenerated from a haploid, as 10.1 percent coefficient of variability for fruit weight. This coefficient of variability should be a fair measure of environmental variation for the character fruit weight, and in comparison, the lower values obtained for corn inbreds on nitrogen forms seem well within the limits of environmental variation expected.

Top-root ratios for corn inbreds showed no relationship with strain response to nitrogen forms. This ratio remained consistent from one nitrogen treatment to another but differed significantly between inbred strains. The significant differences between inbred strains in this ratio clearly illustrates the individuality of these pure genetic strains. Variation in top-root ratio between species has been reported by GOEDEWAAGEN (1937) and CRIST and STOUT (1929).

During the course of this investigation very definite root types were observed in the inbred strains. Such types as the following were characteristic of inbred strains; a thick, heavy, and twisting system; a thick, long, poorly branched system; or a fine shallow system of roots. Such distinct types of root systems are believed to be largely responsible for the differences between strains in top-root ratios.

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SUMMARY

1. Corn inbred strains and single cross hybrids were grown in aqueous mineral solutions supplied with various nitrogen forms. Differential responses by strains and hybrids to ammonium and nitrate nitrogen proved to be statistically significant. Some strains and hybrids made relatively more growth on ammonium nitrogen compared with their growth on nitrate nitrogen than did others.

2. These differential nutritional responses by corn inbreds were due to inherent differences in the genetic constitution of the strains since environmental variation was taken into account. Hybrid responses to nutritional treatments demonstrate the transmissibility of the nutritional complexes present in inbred strains. Partial dominance of the genetic complex for efficient utilization of ammonium nitrogen, or dominance divided between factors for efficiency and for inefficiency is suggested as the mode of inheritance followed by these nutritional characteristics.

3. Percentages of dry matter in tops of corn inbreds grown in solution treatments demonstrate another inherent difference between inbred strains. Not only do strains differ in the percent of dry matter they produce but this factor varies with the nutritional treatment. Those strains which utilized ammonium nitrogen efficiently had little variation in their percent dry matter from one treatment to another. On the other hand, inefficient ammonium-utilizing strains had less dry matter per unit of green weight on ammonium treatment than on nitrate treatment. This is interpreted to signify better metabolism of ammonium nitrogen in efficient ammonium strains than in inefficient ammonium types.

4. Empirical observations of root systems grown in ammonium cultures and dry weight data from two strengths of the ammonium solution agree in showing that strains also differ in their tolerance of ammonium concentration.

5. No association was shown between strains' response to nitrogen form and top-root ratios for strains. Top-root ratios were significantly different between strains. This is in agreement with empirical observations on root systems of inbred strains of corn. Root systems of corn inbreds grown in water cultures are as distinct as are the tops.

6. Tomato strains of two species, *Lycopersicum esculentum* and *L. pimpinellifolium*, were grown on three deficient nutritional solutions (low N, low P, and minus K) and compared with their growth on full nutrient

solution. Significant differential growth was made by strains on each nutritional deficiency.

7. The differential response to potassium levels (full and minus K) by tomato strains was inherited. Hybrid combinations showed equally as striking differential responses as did their parent strains. The genetic complex for efficient utilization of limited potassium supply appears to be complex in nature. At any rate, complete dominance is not present in the F_1 hybrids.

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CONTINUED INBREEDING IN MAIZE

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THE investigation of the effects of inbreeding maize begun by E. M. EAST in 1904 has been continued to the present time. The results of 30 generations of self-fertilization are here summarized. All surviving lines have been reduced to a high degree of uniformity and constancy. Variations of a degenerative nature have occurred from time to time and no doubt will continue to occur. Such variations have caused the extinction of at least one line and may prevent the continuation of some or all of the surviving lines. For the past ten generations however, they have been able to exist at about the same level of vigor without appreciable change.

These inbred lines were started from a number of self-pollinated plants of the Leaming variety grown at the Illinois Agricultural Experiment Station in 1904[1]. This variety was known locally as Chester's Leaming and was selected as one of the most productive types of corn grown in that locality at that time. At least 12 plants were hand pollinated at the start but when the first detailed report on these inbreds was made in 1912 (EAST and HAYES) only four lines had been continued in Connecticut. These were numbers 1–6, 1–7, 1–9, and 1–12.

Number 1–12 was the least productive of the four, yielding only two bushels per acre in 1911 while the others gave 28, 25, and 26 respectively. This 1–12 line was either discontinued or lost sometime between 1911 and 1914. The 1–7 line was separated into two lines in the second generation. One of these, the 1–7–1–1 line, has been continued to the present time. The 1–7–1–2 line was lost in generation twenty for reasons that are not clearly understood. In about generation eighteen several plants showed a peculiar streaking of the leaves. Although seed was saved from only normal plants the condition increased until in generation twenty all of the plants were affected. No selfed seed was secured from a number of pollinations. Attempts to restore the line from old seed were unavailing.

The abnormality seemed to be in the production of chlorophyll. Affected plants were faintly striped or streaked with indistinct light green or yellowish green areas on the leaves. These plants produced pollen but rarely any seed. They were crossed on to normal plants in the same line and in unrelated lines. In both cases abnormal plants appeared in the first and later generations in varying numbers and with different degrees of abnormality.

[1] In 1912, EAST and HAYES stated that the first self-fertilized generations were grown in 1906 from hand-pollinated seed produced in 1905, but an earlier publication by EAST in 1909 presented results which show that the first self-pollinations must have been made in 1904.

In the self-fertilized line the abnormality increased steadily in the proportion of plants affected and the degree to which they were reduced in size and vigor until finally no seed was obtained. This variation has not appeared in any other line.

TABLE 1

The effect of thirty generations of self-fertilization upon the height of plant and yield of grain in maize.

NUMBER OF GENERATIONS SELFED	1–6		1–7		1–9	
	HEIGHT INCHES	YIELD BU. PER ACRE	HEIGHT INCHES	YIELD BU. PER ACRE	HEIGHT INCHES	YIELD BU. PER ACRE
0	117	81±7	117	81±7	117	81±7
1– 5	87	64±11	81	51±7	77	41±5
6–10	97±1	45±12	84±1	36±5	82±2	34±4
11–15	97±3	38±4	84±2	34±3	83±2	26±2
16–20	88±4	22±4	85±3	24±3	75±4	14±3
21–25	81±2	20±6	75±3	21±3	71±3	13±2
26–30	92±3	24±9	80±2	18±4	77±3	9±4

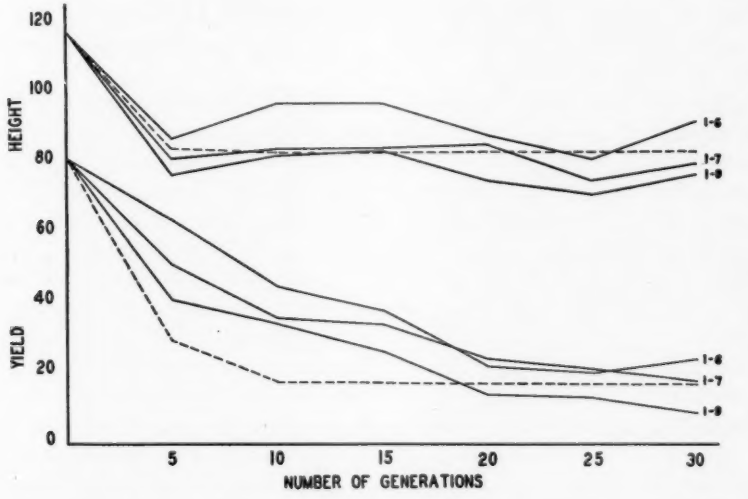

FIGURE 1.—A comparison of three maize lines, derived from the same variety, self-fertilized for 30 generations. Height of stalk is measured in inches and yield of grain in bushels per acre, both plotted on the same scale. The broken lines are the theoretical curves of inbreeding explained in the text.

The other three lines, 1–6, 1–7, and 1–9 have remained normal in every respect except for a much reduced rate of growth. Their behavior as measured by height of stalk and yield of grain during the 30 generations of self-fertilization is given in table 1 and figure 1. The results are averaged

for five-year periods to reduce the seasonal fluctuations. Wide variations, especially in yield, occur from season to season due to varying weather conditions. Since it is impossible to save seed of corn for more than a few years and have it produce normally there is no way to overcome these seasonal variations. A long continued experiment on inbreeding with plants is as much a record of weather conditions as it is of the effects of consanguinity. For example, the 1–6 line in generation thirty was grown both in 1936 and 1938 from the same lot of seed. In the former year it yielded at the rate of 38.3 bushels per acre and in the latter year 1.9 bushels. All yields were high in 1936 and extremely low in 1938 due to the rainy season and late maturity.

No measurements on height of stalk were made until the fifth generation. The original, naturally pollinated variety was grown in 1905 in Illinois and again in 1916 in Connecticut from seed obtained anew from the original source. The yields were 75 bushels of dry shelled grain in Illinois and 88 bushels in Connecticut. These two results are averaged to give the yield at the start before inbreeding. Height of stalk was measured only on the original variety grown in Connecticut in 1916. All yields have been calculated from air dried ears, 70 pounds of ear corn equalling one bushel of shelled grain.

By averaging the results for five-year periods some of the seasonal fluctuations are smoothed out and the general trend stands out clearly. The reduction in height of stalk has taken place almost entirely in the first five-year period. After the fifth generation no significant change has taken place, giving due consideration to the probable error as measured by the standard deviation calculated by Bessel's formula. Yield continued to decline in all three lines until generation twenty. During the last 10 generations no significant change has taken place. The broken lines in figure 1 are the theoretical reductions calculated in the following way: The average height and average yield of the three lines at the end of the 30 generations is subtracted from the figures at the start. This difference is halved in each generation and added to the end result.

In calculating the theoretical curves the results are averaged for five generation periods to correspond with the actual results treated in the same way. Since no height measurements were made until the fifth generation the theoretical height is not averaged for the first five generations. Only the one figure for the fifth generation is used. (Example: $117-83=34$. This number halved five times is 1.0625. Added to 83 it gives the theoretical height for the fifth generation. The theoretical yields for the first five generations, obtained in the same way, are 49, 35, 25, 21 and 19. The average of these is 29.4, the first point in the theoretical curve for yield.)

These expectations are based upon the assumption that the inbreeding

effect is correlated with the degree of heterozygosity as explained previously (JONES 1918a). The number of heterozygous allelic pairs is reduced 50 percent, on the average, in each generation of self-fertilization. The more rapid attainment of constancy in height than in yield may be understood on the assumption that height of stalk is less complex in its transmission than yield. Grain production sums up the plant's entire ability to grow and to reproduce and no doubt is influenced in its expression by many more inherited determiners than length and number of internodes.

In a previous publication (JONES 1924) results were given from two of these Leaming inbreds which were crossed, and successive selfed generations from the F_1 to F_8 grown, all in the same years. New seed was produced from a number of plants self-fertilized. Since the original plants were first generation hybrids of inbred strains the results are not directly comparable to the figures given above. For testing the effect of inbreeding upon yield they are better because seasonal fluctuations are eliminated to a considerable extent and the mixing of seed from several plants reduces the chances of using progenitors more heterozygous than the average. The yields in bushels per acre obtained compared to the theoretical expectations are as follows:

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Actual	101	69	43	44	23	27	25	27
Expected	—	64	46	36	32	29	28	—

This is a remarkably close parallel considering the many external factors that can affect yield. The height measurements obtained from the same plants are not so closely in agreement with the theoretical expectation. They are as follows:

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Actual	95	82	78	77	67	63	60	59
Expected	—	77	68	64	61	60	60	—

The slower reduction in any measurable character, than the theoretical curves indicate, could result from the survival and selection of the more vigorous individuals as progenitors. In this way the attainment of homozygosity is delayed. It is expected that inbred lines will behave differently. By random assortment some individual progenitors will be more heterozygous and some less than the average. Experience with a large number of inbreds indicates that the majority of them are reduced to a high degree of stability in less than ten generations.

Since the standard errors are so high there is no certainty that the reduction in yield and height after 30 generations has come to an end. Moreover this reduction may go on indefinitely even if the plants are now re-

duced to complete homozygosity, due to degenerative changes. There is good evidence to indicate that loss variations have occurred in these lines. In figure 2 the behavior of two lines is given when separated at various

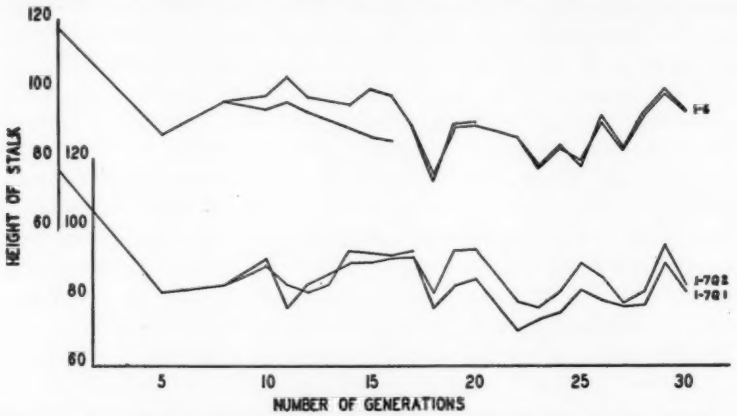

FIGURE 2.—Two families separated into sib lines at various generations and continued until shown to be genetically similar or diverse. Both are plotted at the same scale in different positions.

stages of inbreeding. Height of stalk is used because it is less influenced by seasonal fluctuations than yield. The 1–6 line in the eighth generation was separated into two sib lines and grown in adjoining rows until the sixteenth generation. The two lines were clearly distinct as reported in a previous publication (JONES 1924). At the same time the 1–7 line was also separated and the two sib lines continued to the 17th generation.

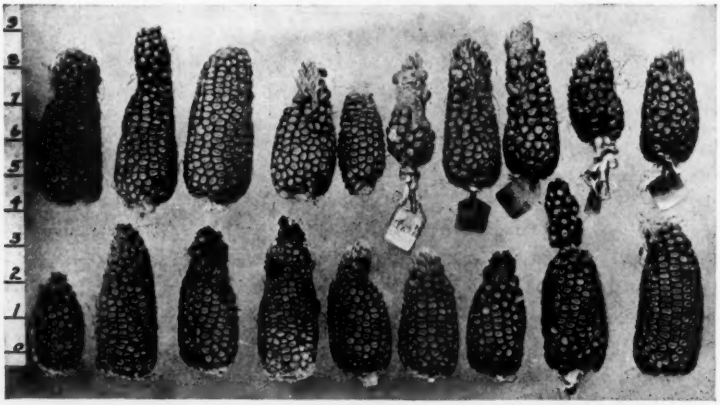

FIGURE 3.—The 1-7Q1 line in the 29th generation of continuous self-fertilization.

As no significant difference appeared in these paired lines one of them was discontinued and another separation made in the other in generation 17. These two lines 1–7Q1 and 1–7Q2 have been continued to date and have been significantly different for this entire period in appearance as well as in measurable characters. The difference in ear type is shown in figures 3 and 4. The 1–7Q2 line has noticeably smaller and less well filled

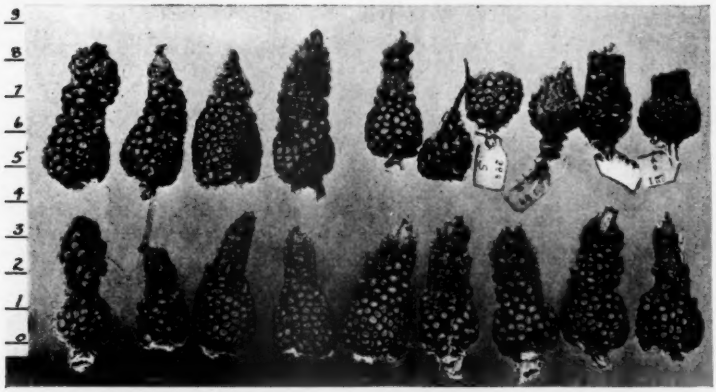

FIGURE 4.—The 1-7Q2 line separated from the 1-7Q1 line in the 17th generation and further inbred for 12 generations.

ears. The production of grain is significantly less as shown by the results given in table 2. This poorer yielding line is taller in stalk growth. The

TABLE 2

Behavior of inbred lines of maize separated in the 17th generation and continued to the 30th generation of self-fertilization.

GENERATIONS SELFED	HEIGHT OF STALK—INCHES			YIELD—BUSHELS PER ACRE		
	1–7Q1	1–7Q2	DIFF. 1–2	1–7Q1	1–7Q2	DIFF. 1–2
18	77.3	80.5	−3.2	—	—	—
19	82.6	92.5	−9.9	—	—	—
20	84.4	93.4	−9.0	19.1	17.8	+1.3
22	70.1	78.0	−7.9	14.1	9.6	+4.5
23	73.0	76.5	−3.5	19.8	21.4	−1.6
24	74.8	81.3	−6.5	23.5	15.9	+7.6
25	81.8	89.7	−7.9	27.3	22.2	+5.1
26	79.1	85.9	−6.8	22.9	14.0	+8.9
27	76.6	77.9	−1.3	19.5	16.1	+3.4
28	76.7	81.3	−4.6	26.7	16.9	+9.8
29	88.6	94.2	−5.6	16.9	10.1	+6.8
30 1936	80.9	82.6	−1.7	31.3	25.3	+6.0
30 1938	75.6	73.4	+2.2	4.2	2.5	+1.7
Average	78.6	83.6	−5.0	20.5	15.6	+4.9

differences in height are all minus and in yield all plus, with one exception in each case, and the odds measured by Student's method are considerably less than one in 100 that these differences are due to chance alone.

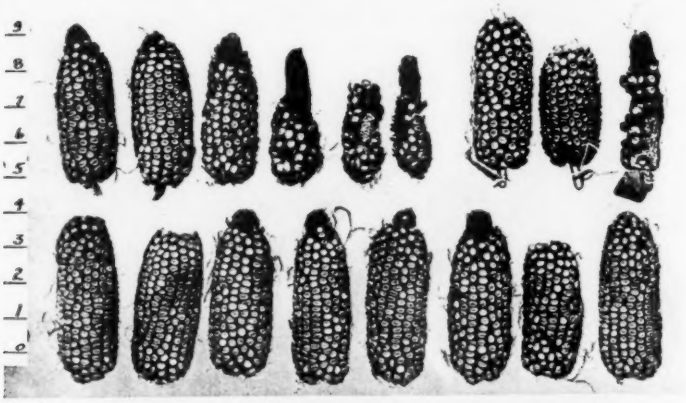

FIGURE 5.—The 1-6V1 line in the 29th generation of continuous self-fertilization.

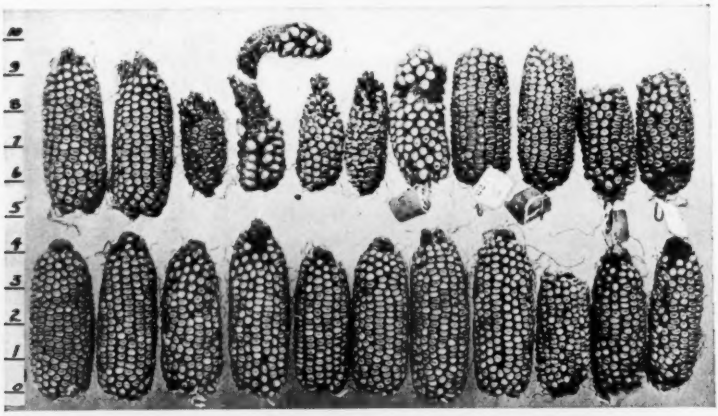

FIGURE 6.—The 1-6V2 line separated from the 1-6V1 line in the 22nd generation and further inbred for seven generations.

The 1–6 line was also again separated in the 17th generation but as no differences were apparent in generation 20 one of these was discontinued. It was again separated in generation 22 and the two sib lines continued to date. Height measurements made in the 28th generation are given in table 3 and show a slight difference which is not statistically significant. Representative ears of these two sib lines are shown in figures 5 and 6.

A sensitive method for testing heterozygosity is to cross separated lines and compare the F_1 progeny with the two parental lines. This was done for all three lines separated after seven generations and continued for six or more additional generations (JONES 1924). The crosses of the paired lines were significantly increased in yield over the parents in the 1–6 and 1–7 lines but not in the 1–9 line. Sib lines of 1–6 and 1–7 separated in the 22nd and 17th generations and further self-fertilized for six and eleven generations were crossed with each other and the results for height and yield are given in table 3. The F_1 progenies do not differ significantly from

TABLE 3

The result of crossing inbred lines of maize that had been separated in generations 17 and 22 and further inbred for 11 and 6 generations.

PEDIGREE	HEIGHT OF STALK IN INCHES										N	MEAN	YIELD S.D.	BU. PER ACRE	S.D.
	61	64	67	70	73	76	79	82	85	88					
1-7Q1 K	8	5	10	2							25	65	3.0	27	3.8
F_1	1	13	19	12	3						48	67	2.8	25	1.2
1-7Q2 K		3	9	4	6	1					23	69	3.1	17	2.5
1-6V1 F					2	7	8	7	3	1	28	80	3.7	36	2.5
F_1				1	3	11	16	7	3		41	79	3.3	33	1.4
1-6V2 F				1	5	4	9	8	1		28	78	3.7	35	1.9

either parent. There is every indication that these lines have now been reduced to complete, or very nearly complete, homozygosity, at least for all loci that have any effect upon hybrid vigor.

The differences that have appeared in the last 15 generations are more probably due to germinal variations rather that to delayed segregation from an originally heterozygous complex. Two transmissable variations had previously appeared in these lines. Defective seeds were noted in the 1–6 line in the 13th generation and red cobs in the 1–7 line in about the tenth generation. This latter is a change from a recessive to a dominant condition.

Since these inbred strains are so uniform in all visible characters and so much reduced in size and vigor, any outcrossing which may take place is immediately noted in the much larger and more vigorous plants which result. None of the variations noted can be attributed to contamination.

The 1–6 line was the most vigorous and high yielding of all the lines grown in the first half of the inbreeding period and at the present time is the most productive. Representative ears of this line are shown in figures 5 and 6. Comparing these specimens with the ones shown in figure 7 for the seventh generation and in Connecticut Agricultural Experiment Station Bulletin 207, Plate IIa for the tenth generation it will be seen that

the ears are now somewhat shorter than they were in the early stages of inbreeding. Ears of this line in about generation 13 are shown in GENETICS 9: 405–418, 1924, figure 1. These are quite similar to the ones shown here produced in generation 29. (Previous photographs show selected ears. The illustrations given here include the entire crop from small progenies of about 20 plants each. Some of the small specimens are second ears.) In stalk growth and kernel type there has been no marked change since the tenth generation. This line now produces much aborted pollen apparently due to a failure of normal synapsis.

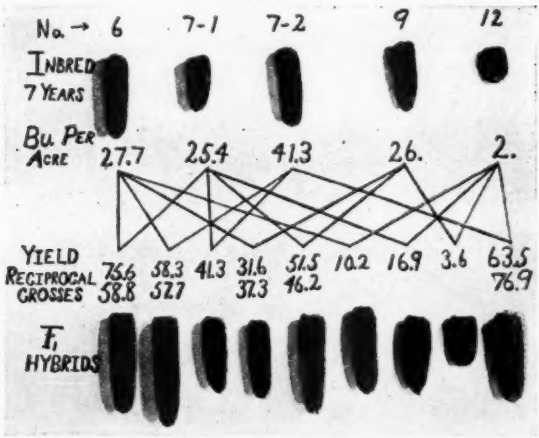

FIGURE 7.—The four lines after seven generations of self-fertilization. (From photograph by H. K. HAYES taken in 1914)

The 1–7 line shown in figure 3 is considerably smaller in ear size than it was in generation 10. There is a noticeable tendency for the ears to be poorly filled at the tips with the production of many rudimentary staminate florets. While the cobs are still flattened they are not as broad as the ears shown in Bulletin 207, Plate IIb. The change in height of plant and ear type, shown by the sib lines separated in generation 17, is a germinal change of some kind. The reduction in size of ear and seed formation would be interpreted as a degenerative change if it were not that the plants are taller than the normal line which has not changed in ear type. Whether these changes involve more than a single locus has not been determined.

The 1–9 line has been the least productive of the three. It is a heavy pollen producer but is so late in maturing that its yield is often cut short by the end of the growing season. In 1938 it yielded practically nothing. No

ears are available for photographing and only a few immature self-pollinated seeds are on hand to continue this line. In 1937 it yielded at the rate of 21 bushels per acre which is considerably above its average for the past 15 years. In a good year the ears do not differ noticeably from the specimens shown in Bulletin 207, Plate IIb. (See also Inbreeding and Outbreeding, p. 130.) Ears of this line in about the fifth generation are shown in United States Department of Agriculture Bulletin 243, Plates III and IV, and in the seventh generation in figure 7. From the fifth to tenth generation there had been a remarkable change.

TABLE 4

The behavior of three inbred lines of maize with respect to infection by the smut fungus (Ustilago zeae) *from generation 10 to 30.*

YEAR GROWN	PERCENT OF PLANTS INFECTED					
	1–6		1–7		1–9	
1917	0		9.8		0.3	
1920	10.0		13.8		5.7	
1922	2.2		27.3		3.0	
1923	7.4		69.0		0	
1925	2.9		19.6		0.7	
Average		4.5		27.9		1.9
1926	5.0		46.5		0	
1928	5.0		63.0		0	
1929	0		15.7		0	
1930	0		52.4		5.6	
1931	21.7		56.8		29.8	
Average		6.3		46.9		7.1
1932	7.1		71.4		11.4	
1933	3.9		43.6		18.3	
1935	29.2		83.9		11.1	
1936	0		11.3		—	
1937	—		—		45.0	
1938	3.8		24.2		0	
Average		8.8		46.9		17.1
Total Average	6.5		40.6		8.7	

In the tenth generation it was noted that these inbred lines differed in susceptibility to the smut fungus (*Ustilago zeae*). A preliminary report was made in 1918b. In table 4 are given the percent of plants infected from generations 10 to 30. The figures are arranged according to the years grown and not by generations since the fluctuation was mainly seasonal. Comparing the averages for five year periods there has been an increase in number of plants affected. The relative position of the three lines has remained about the same. The 1–7 line from the start has been the most severely injured. Not only do more plants of this line show the black fruit-

ing bodies of the fungus but these spore-bearing structures are larger and the plants show more injury. There are many barren stalks and some plants are killed before maturity. In this line the black spore masses usually appear at the nodes near the base of the plant or at the ear nodes. In the other two lines the fruiting bodies appear in the tassel or at the upper nodes. In these lines also the smut balls are usually small and the plants are seldom injured appreciably.

The increase in percent of plants infected seems to be one of the manifestations of the effect of inbreeding. As the plants are reduced in vigor they are less resistant. Since the increase in susceptibility has come after generation 20 at which time the reduction in yield is assumed to have come to an end this may not be a valid conclusion. The wide fluctuation in number of plants affected is largely seasonal. The years from 1931–1937 were a period of relatively high smut infestation.

No variations during the long continued inbreeding program have appeared that can be considered as favorable to survival. Usually not more than 100 plants of any one progeny have been grown each year, not a large number in which to look for variations. But many progenies have been grown and in the 10 years after uniformity and constancy had been reached there has been a reasonable chance for growth variations to appear. Because the plants are so uniform, slight variations would be noticeable and anything favoring growth would likely be selected. Since these inbred strains are so late in maturing any variation that helped the plants to ripen earlier would tend to be perpetuated. Possibly unconscious selection of favorable growth variations has counteracted to some extent the reduction due to inbreeding. But if there is any tendency of this kind it did not save two lines from extinction and there is no certain indication that any of the lines are now improving in any character.

SUMMARY

From four original lines of maize three have been continuously self-fertilized for 30 generations.

One original line and one sub-line, separated in the second generation, failed to survive. At least in one case this failure is due to a degenerative change which apparently could not be prevented by selection of normal plants as progenitors.

Reduction in height has ceased after five generations and in yield after 20 generations.

Sib lines separated at various stages clearly differed in some cases and remained the same in others.

These differences are considered to be the result of spontaneous transmissable variations and not the result of delayed segregation.

After 20 generations of self-fertilization these inbred lines appear to be uniform and constant for all visible characters and homozygous for all loci that have any effect upon hybrid vigor.

Throughout the entire period of inbreeding no variations have appeared which could be interpreted as being favorable to survival.

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THE GENETICS AND PHYSIOLOGY OF LETHAL ANEMIA IN THE RAT[1]

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INTRODUCTION AND LITERATURE

THE "anemic" mutation appeared in the Cornell colony of albino rats which originated from the Osborn and Mendel stock at the Connecticut Agricultural Experiment Station. Since 1919 the Cornell colony has been a closed one, but inbreeding has not been intense. The rats are maintained on an adequate mixed diet (a commercial calf meal) which is supplemented at least once a week with cod liver oil to the extent of three percent of the diet (MAYNARD 1930). The anemia appears spontaneously in the young rats and acts as a complete lethal, since all of the affected animals die, usually within the first two weeks of life. When first observed by the authors the gene was so widespread in the colony that it was impossible to trace it back to any one or two individuals in which it may have first occurred. That the mutation occurred in the Cornell colony is supported by the fact that no anemic animals have been noted in the Osborn and Mendel colony (private communication).

CREW and KON (1933) found a monogenic autosomal lethal in the rat which first manifested itself at about the 9–12th day of life when the young rats began to lose weight. They died usually within five days from inanition. The underlying cause of this failure could not be determined, but the authors stated that neither an anemia nor a physiological leucocytosis was present.

GRÜNEBERG (1938), in describing another simple recessive autosomal lethal in the rat, says, "The most fundamental disturbance so far discovered is an anomaly of the cartilage; this simple mechanism affects several structures of the body in a similar way. Hyperplasia of the ribs and of the cartilage rings in the trachea thus produced leads secondarily to the development of an emphysema of the lungs, and the animals ultimately die from more or less remote consequences of this emphysema." The time of death of these rats varied from 4–39 days of age.

GUNN'S (1937, 1938) case of acholuric jaundice in the rat is another example of a hereditary physiological upset. This is due to an incompletely recessive autosomal gene, the heterozygotes showing some of the characteristic symptoms of the variation, such as increased red cell fragility and reticulocytosis. This mutation is not incompatible with life, for the affected

[1] Supported in part by a grant from the Rockefeller Foundation for the study of longevity.
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animals live and can reproduce. It is characterized by a series of symptoms consisting of increased red cell fragility, bilirubinemia, microcytosis, reticulocytosis, splenomegaly, jaundice and anemia, which are pronounced in the young growing animals.

There are well established cases of hereditary anemias in mice, among which is the anemia associated with the "dominant white spotting" factor (*W*) reported by DE ABERLE (1925a, 1925b, 1927) and DE ABERLE, HOSKINS and BODANSKY (1927). The anemia appears in the homozygous dominant individuals (*WW*) and causes death within a week or ten days. According to DE ABERLE, the anemia is first manifested in the (*WW*) mouse at about the 16th day of intra-uterine life, at which time the hemopoietic cells of the spleen and liver sinuses are deficient in number. The appearance of this anemia thus occurs when the bone marrow is undergoing organization for future hemopoiesis. DE ABERLE stated that the anemia is of an aplastic type and that affected animals have about 25 percent as much hemoglobin and 14 percent as many red cells as the normal mice. These anemic mice tend toward a leucopenia, with about 20 percent as many leucocytes at birth as the normals.

Another case of anemia in mice is that associated with the autosomal recessive flexed-tailed gene first described by HUNT and PERMAR (1928). The associated anemia was first observed by MIXTER and HUNT (1933) and reported on further by KAMENOFF (1935). According to the latter author it appears at about the 14th day after fertilization. It thus precedes the time when the bone marrow begins to take over the hemopoietic function, which in the normal mouse embryo is at the 16th day. The erythrocyte counts in the flexed-tailed mice up to the time of birth are between 50 and 75 percent that of the normals for this period. The anemia persists in the flexed-tailed mice for about two weeks after birth; the blood then becomes normal. ANDERSON (cited by KAMENOFF) has traced the defect to faulty hemopoiesis in the liver of the embryo where the percentage of normoblasts was significantly less and the percentage of erythroblasts was significantly greater than in normal mice.

DUNN (1937) mentions that mice homozygous for the fused gene ($T^t\,T^t$) possess a lowered viability and may have an embryonic anemia.

The occurrence of the anemia described in this paper was reported in a preliminary note by BOGART, SMITH and KIMBALL (1938). This paper greatly extends the observations which were reported at that time.

DESCRIPTION OF THE ANEMIC RATS

This anemia is first noted at about 2–3 days of age at which time the anemic young acquires a pallor that becomes more pronounced so that in a day or two the afflicted rat is easily distinguished from its normal sibs.

These affected animals are usually dead within two weeks. The oldest anemic rat so far recorded survived to only 29 days of age; therefore, this character is a complete lethal. Paralleling the progress of the "anemic appearance" is a jaundice which gives the rat a yellow color that is noted on the exterior and in the internal organs; particularly in the liver and intestinal tract.

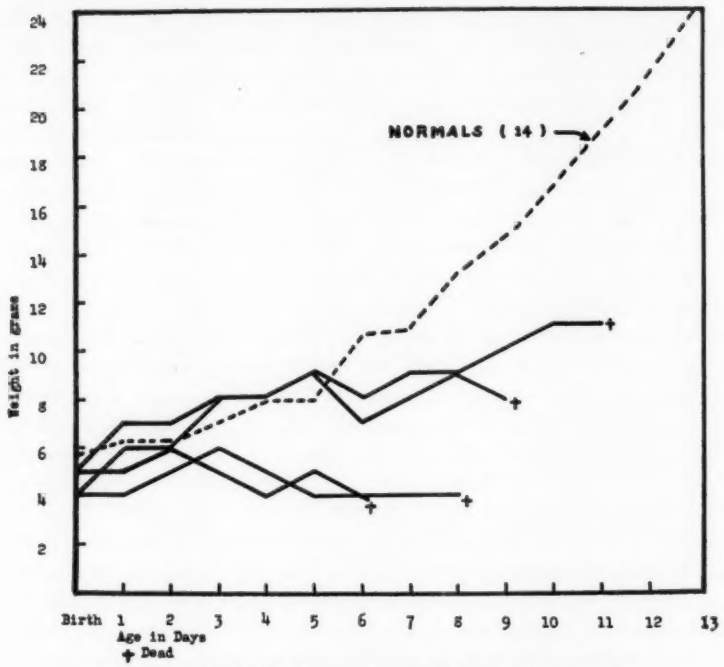


FIGURE 1.—Comparison of growth curves of normal and anemic rats.

The growth of normal and anemic littermates is given in figure 1. The anemic individuals decrease in weight, beginning about 3–5 days after birth and usually continue to lose weight until they die.

Nine anemic and 9 normal young (littermate pairs) ranging from 6–8 days of age were preserved in a 10 percent formalin solution. The hearts, liver, spleen and kidneys were then carefully dissected out, blotted on a filter paper, and weighed to the nearest hundredth of a gram. The difference in the normal and anemic rats did not show a mathematical significance for heart and spleen weights. Although the anemic and normal rats were the same age (littermates), the size in the two groups differed markedly. The anemic young were only a little over half the size of their

normal sibs. The livers and kidneys were actually much smaller, but in relation to live weight of the young rat considerably larger in the anemic rats (table 1). Thus, when the actual weights are considered, the livers and kidneys are significantly larger (odds 26:1 for livers and 400:1 for kidneys) in the normal rats, but when based on percentage of live weight, these organs were significantly larger in the anemic rats. It is difficult to determine whether the difference in sizes of the livers and kidneys is due to the anemia itself or to the interruption of growth which was brought about by the anemia.

TABLE 1

A comparison of organ weights of normal and anemic rats.

	AGE IN DAYS	NO.	LIVE WT. (GMS)	HEART (GMS)	LIVER (GMS)	SPLEEN (GMS)	KIDNEY (GMS)
Normal	6–8	9	11.6	.10 ± .007	.36 ± .02	.05 ± .006	.14 ± .006
Anemic sibs	6–8	9	6.6	.09 ± .005	.28 ± .02	.04 ± .005	.10 ± .005

Some attempts have been made to cure the disease, but so far these have all proven fruitless. Liver[3] extract was both fed and injected intraperitoneally in doses as high as .1 ml but without results. A solution of iron sulphate with a trace of copper sulphate was fed to pregnant females and to the young, and still the anemia appeared. To determine whether or not the rat anemia might be due to the infectious *Bartonella muris* organism (ELIOT and FORD 1929), blood of anemic rats was injected into young normal rats, but the results were negative. A study of the blood smears of anemics also failed to show the presence of Bartonella bodies.

GENETICS

The lethal acts as a simple autosomal recessive; the heterozygotes (*An an*) do not present any of the anemic characteristics. No evidence for a sex influence has been obtained.

The results of mating together individuals that had previously produced some anemic offspring were very close to a 3:1 ratio (table 2). This would in itself indicate the recessive nature of the gene. The data given in table 2 show that the sex ratios in both the normal and the anemic offspring do not deviate significantly from the expected 1:1 ratio.

Because of the lethal nature of the gene, it was necessary to use known heterozygous animals for testing the genotype of other individuals. Any animal that produced anemic offspring was considered to be heterozygous, but, if 25 or more normal offspring were produced by an individual that was mated to a known heterozygote, it was assumed that the animal tested was homozygous for the normal condition.

[3] The liver extract was a product of the Eli Lilly Company.

The genotype of the normal offspring resulting from the mating of heterozygous animals *inter se* was tested by mating to one or more known heterozygotes. A total of 85 individuals (19 males and 66 females) was tested. Twenty-eight proved to be homozygous and 57 heterozygous (table 2). These results closely fit the 2:1 ratio, which would be expected if only one pair of alleles is involved. The heterozygotes were evenly distributed between the sexes.

The genotypes of the offspring resulting from the mating of *An An* males to *An an* females and from the reciprocal mating were tested according to the procedure just outlined. A total of 26 individuals—7 females and 19 males—was used. Fourteen were found to be *An An* and 12 *An an* (table 2). There was no discrepancy between the two sexes, although the number of observations was small. The sex of the heterozygous parent did not influence the ratio of homozygous to heterozygous offspring. These results again indicate that only one pair of autosomal alleles is involved.

Because of the possibility that complementary genes might be responsible for the anemic condition, the genotype of ten offspring resulting from the mating of *An An* together was ascertained. In every case these animals proved to be homozygous (table 2). Although the number of individuals tested was not large, the results indicate that complementary genes were not responsible for the anemic condition.

TABLE 2

The results of various matings are shown herein.

	NORMAL				ANEMIC				RATIO	
	TOTAL	MALES	FEMALES	PERCENT	TOTAL	MALES	FEMALES	PERCENT	OBTAINED	EXPECTED
(*An an*) mated together	534	268	266	74.9	179	87	92	25.1	2.98:1	3:1

	An an	*An An*	RATIO	
			OBTAINED	EXPECTED
Genotype of the littermates of the anemics. 85 animals (19♂♂ and 66♀♀) tested	57	28	2.04:1	2:1
Genotype of offspring (*An an*×*An An*)	12	14	1:1.17	1:1
Genotype of offspring (*An An*×*An An*)	0	10	All homozygotes	All homozygotes

In order to determine if *An* is completely dominant over *an*, the hemoglobin was determined in some adult female rats that were of the genotypes *An an* and *An An*. The two groups were not significantly different

and the *An an* females possessed just as much hemoglobin as the *An An* females (table 3).

TABLE 3

A comparison of the hemoglobin of homozygous and heterozygous adult female rats.

GENOTYPE	NUMBER	GRAMS OF HEMOGLOBIN PER 100 ML OF BLOOD
An An	10	14.57±.27*
An an	11	14.42±.38

* Standard errors are used throughout this paper.

More information which indicates that the heterozygous animals are not adversely affected by the presence of *an* is given in table 4. These data were secured from an experiment designed to study the effects of various reproductive regimes on the life span of rats. It was later learned that *an* gene was widely dispersed among the rats in this experiment. Inasmuch as this experiment was not primarily designed to study the genetics of anemia, a complete analysis of the genotypes of the rats involved was not available, but several proved to be heterozygous for anemia. All known heterozygous females were grouped and their performance was compared to the breeding group as a whole, which was thus made up of homozygous and heterozygous females. One observes from the table that, to date, the heterozygous females did not differ in comparison to the total group insofar as span of life and fertility are concerned; in fact, the data indicate that the heterozygous females may be a little superior to the homozygous females in these respects.

TABLE 4

A comparison of the reproductive performance of homozygous and heterozygous normal female rats.

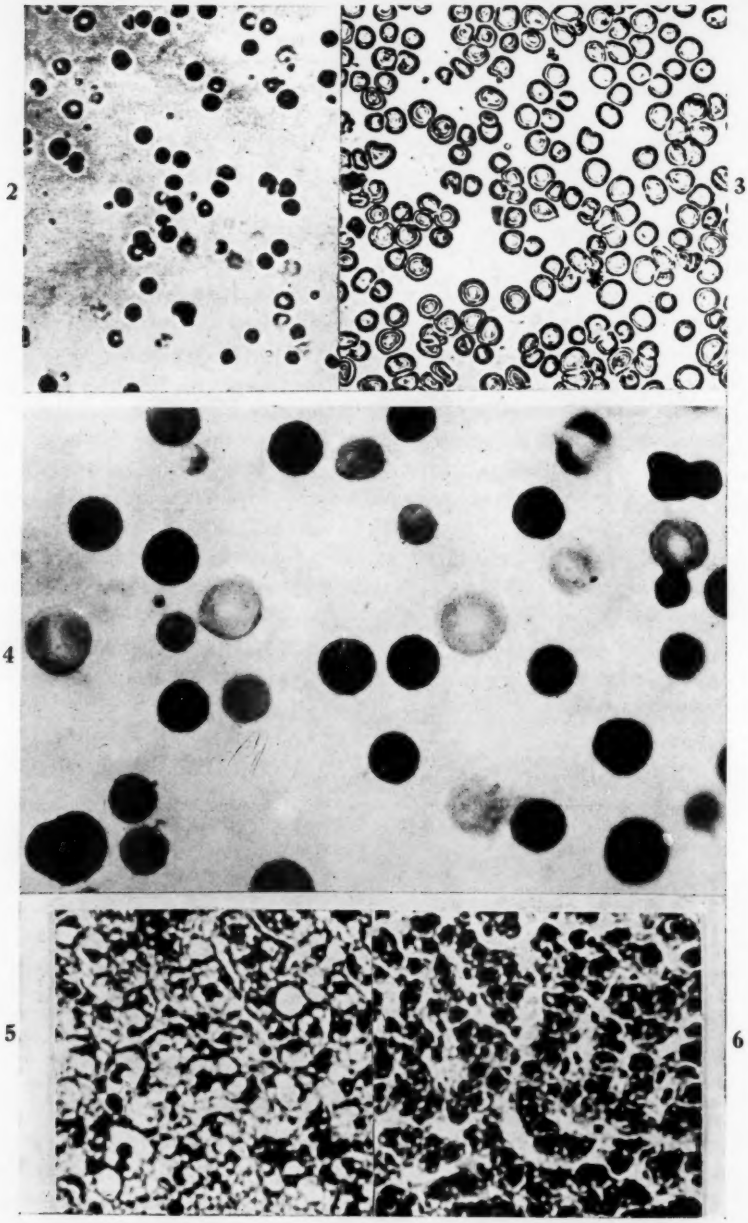
GROUP		NUMBER	PERCENT DEAD TO DATE	AVERAGE LITTERS PER FEMALE	AVERAGE YOUNG PER FEMALE	AVERAGE LITTER SIZE
Bred early	Total	50	26.0	7.9	49.8	6.3
	An an	13	7.7	10.1	63.3	6.3
Bred normal	Total	50	36.0	8.9	53.1	6.0
	An an	12	33.0	8.0	53.0	6.6

Bred early group—delivered the first litter at an average age of 87 days.
Bred normal group—delivered the first litter at an average age of 129 days.

HEMATOLOGY AND HISTOLOGY

1. General morphology of the red cells

Smears were made from blood secured by decapitating the rats and were stained with Wright's alkaline methylene blue and with Giemsa's stain



FIGURES 2–6. (See opposite page for description.)

buffered at pH 6.7 with a phosphate buffer. The erythrocytes of the anemic rats show a marked anisocytosis (variation in red-cell size) due to the presence of microcytes which, microscopically, do not show the concavity of the normal red cells and thus are probably spherocytes (table 5 and figures 2 and 3). The measurements were made by projecting at a magnification of 4,600× and measuring to the closest mm, and the data calculated to micra. There is a slight poikilocytosis and a marked polychromasia (variation in staining ability) of the erythrocytes of the anemic young (figure 4). In addition, the anemic smears contain single and multiple Howell-Jolly bodies and a few nucleated red cells.

TABLE 5

Measurements of red blood cells of anemic and normal 5–9 day old rats.

ANIMAL	NO.	FREQUENCY OF CELLS IN PERCENT								LARGEST	MEAN
		BELOW 3.3μ	3.3 4.3μ	4.3 5.4μ	5.4 6.5μ	6.5 7.6μ	7.6 8.7μ	8.7μ AND OVER	SMALLEST		
Anemics	8	1.7	12.3	37.3	30.9	15.2	2.2	0.4	1.5μ	10.4μ	5.4± .06μ
Normals	7			3.2	18.2	41.2	28.2	9.2	4.3μ	10.4μ	7.2± .06μ

2. Erythrocyte counts and hemoglobin determinations; color index

The red cell counts were made by the usual technics, but instead of using the hematin method the hemoglobin was determined by the iron analysis of the blood. The method of KENNEDY (1927) was used in preference to the easier method of WONG because the former gives truer iron values according to KLEIN (1931). BARCROFT (1928) has pointed out that the hemoglobin of various animals has an iron content of .335 percent. Therefore, the milligrams of iron per 100 ml blood multiplied by the factor .299 gives the grams of hemoglobin per 100 ml of blood. The presence of some non-hemoglobin iron would give an error, but JENKINS and THOMPSON (1937) have shown that in man not more than ten percent of the total iron is of this nature. The error would be very slight in this problem be-

EXPLANATION OF FIGURES 2–6

FIGURE 2.—Blood smear from a 7-day old anemic rat. Compare with fig. 3, which was taken at the same magnification. Microcytes, which are spherocytes, and polychromasia are evident.

FIGURE 3.—Blood smear from a 7-day old normal rat. Note the absence of polychromasia and that these cells are not spherocytes. Compare with figure 2.

FIGURE 4.—Red blood cells from a 7-day old anemic rat, highly magnified. Polychromasia, Howell-Jolly bodies, and spherocytes are striking in this picture.

FIGURE 5.—Section of liver from a 7-day old anemic rat. Bouin, H. and E. technic. Note vacuolar appearance, which is probably due to fat deposits that were washed out.

FIGURE 6.—Sections of liver from a 7-day old normal rat. Bouin, H. and E. technic. Compare with figure 5.

cause the normal and anemic rats are compared. There could be a difference in the percentage of non-hemoglobin iron in the two groups of rats, however. This point has not been investigated. The color index which expresses the amount of hemoglobin per red cell of the anemic relative to the normal is calculated:

$$\frac{\dfrac{\text{Hb of anemic}}{\text{Hb of normal}} \times 100}{\dfrac{\text{R.B.C. of anemic}}{\text{R.B.C. of normal}} \times 100} = \text{Color index.}$$

The color indices of some anemic rats are given in table 6. With the exception of two individuals that are high, the indices approximate unity, indicating that the hemoglobin per red cell of the anemic rats is the same as that of the normal.

TABLE 6

Color index of the anemic rats.

AGE IN DAYS	% ERYTHROCYTES OF THE NORMAL	% HEMOGLOBIN OF THE NORMAL	COLOR INDEX
7	37.9	35.8	0.94
7	42.9	38.9	0.91
7	38.8	36.6	0.94
7	41.5	43.5	1.05
7	30.5	32.5	1.07
7	28.8	26.9	0.93
7	25.3	29.6	1.17
7	45.8	59.2	1.29
7	21.3	35.8	1.68
7	33.8	35.8	1.06
7	16.3	25.7	1.58
8	39.5	40.8	1.03
Total 12	Ave. 33.5	36.7	1.10

The anemic rats have only one-third the normal number of red cells (table 7). Our average red cell count of 2,913,000 obtained from 25 normal rats of 7–8 days of age is in agreement with JOLLY (1909) and KINDRED and COREY (1930).

TABLE 7

Erythrocyte counts of normal and anemic rat blood.

GROUP	AGE IN DAYS	NUMBER	AV. ERYTHROCYTES PER CMM OF BLOOD	COEFFICIENT OF VARIABILITY (%)
Normal	7–8	25	2,913,000±47,000	8.1
Anemic	7–8	12	977,000±77,000	27.4

The hemoglobin content of anemic rat blood averages slightly more than one-third that of normal littermates (table 8). The average value of 10.7 gms hemoglobin per 100 ml blood for our normal rats of 5–9 days of age is in agreement with JOLLY (1909) and WILLIAMSON (1926), although these workers used different methods. The average hemoglobin value of 14.5 gms per 100 ml of blood (table 3) for 21 adult rats is in agreement with the figures of KINDRED and COREY (DOWNEY 1938) and WILLIAMSON (1926).

TABLE 8

Hemoglobin content of normal and anemic rat blood.

GROUP	AGE IN DAYS	NUMBER	AV. GMS HEMOGLOBIN PER 100 ML OF BLOOD	COEFFICIENT OF VARIABILITY (%)
Normal	5–9	25	10.71 ± .30	14.0
Anemic	7–8	12	3.93 ± .27	23.8

3. Leucocyte and differential white cell counts

There is a fourfold increase in the number of leucocytes in the blood of anemic rats (table 9). When the leucocytosis was analyzed in more

TABLE 9

Leucocyte counts of normal and anemic blood.

GROUP	AGE IN DAYS	NUMBER	AV. LEUCOCYTES PER CMM BLOOD	COEFFICIENT OF VARIABILITY (%)
Normal	5–9	11	2,700 ± 200	24.0
Anemic	5–9	7	10,000 ± 1,300	34.6

detail by differential counts (table 10), the polymorphonuclear neutrophile was the principal type of cell to show an increase. On a relative basis there was a doubling of the percentage of neutrophiles in the anemics as compared to the normals, and there was a drop of about one-third in the mononuclear cells (lymphocytes and monocytes). However, on an absolute basis, there was an average eightfold increase of the neutrophiles and a twofold increase of the mononuclear cells (table 10).

TABLE 10

Differential white cell counts of anemic and normal rats.

GROUP	NO.	POLYMORPHONUCLEAR CELLS			MONONUCLEAR CELLS
		BASOPHILES	EOSINOPHILES	NEUTROPHILES	(LYMPHOCYTES AND MONOCYTES)
Normal	4	nil	0.2%	26.3%	73.5%
Anemic	3	nil	0.4%	56.3%	43.3%

The average leucocyte count of 2,700 per cmm of blood for normal rats of 5–9 days of age is in agreement with the values reported by KINDRED and COREY (1930) and JOLLY (1909).

4. Reticulocyte enumeration

Reticulocytes were counted from dry smears of supravitally stained blood cells. A drop of blood in one ml of staining solution made up of equal parts of .5 percent sodium chloride and 2.5 percent sodium citrate plus enough brilliant cresyl blue to give a deep blue color was incubated for one hour at 37.5°C. This tube containing the solution was then centrifuged lightly and the supernatant fluid poured off. A smear was made from the residue in the bottom of the tube and the reticulocytes counted under oil. A total of 500 cells was counted to arrive at the percentage of reticulocytes. The anemic rats have only about one-sixth the normal number of reticulocytes. The normal young ranged from 15–43 percent reticulocytes in their blood, whereas the anemics ranged from 1–10 percent (table 11). A high reticulocyte count indicates that the erythropoietic organs (bone marrow and spleen in the rat) are able to respond to an abnormal need for more blood cells. Comparative absence of reticulocytes in man usually indicates an aplasia of the erythropoietic system. The normal young have an anemia which is due to their iron-poor diet of milk. This milk anemia calls forth a reticulocytosis giving the young rat a count of 25–50 percent as compared to the adult count of 2–4 percent.

The clotting time of the anemic blood is normal. This fact, combined with other characteristic symptoms, differentiates this blood abnormality from that caused by a deficiency of vitamin K in the rat (GREAVES and SCHMIDT 1937).

TABLE 11

Reticulocyte counts of normal and anemic rat blood.

GROUP	AGE IN DAYS	NUMBER	AVERAGE PERCENT RETICULOCYTES	COEFFICIENT OF VARIABILITY (%)
Normal	5–9	17	31.6±2.1	27.4
Anemic	5–9	10	5.3±0.9	53.7

5. Histological studies

Free iron in various tissues. The Prussian blue method was used for this qualitative study (LEE 1937). The livers of every anemic rat studied (9) showed an abundance of free iron primarily in the von Küpffer cells, whereas only traces of free iron could be found in the livers of normal rats. Traces of free iron were found in the splenic capsule of the anemic rats, but there was none in the spleen of the normal rats. Such tissues as skin,

kidneys, intestine, and stomach gave no positive test for free iron in either group of rats.

Fat deposition in liver tissue. The ordinary Bouin's fixed liver material stained with hematoxylin and eosin showed a marked difference between the anemic and normal rats. The liver cells of the anemic rats were vacuolated in appearance following this technic (figures 5 and 6) and indicated that fat material which was originally in these cells had been dissolved out. In an osmic acid fixative, the fat was blackened and preserved. A tremendous fatty deposit was present in the liver cells of the anemic rats, whereas only small and scattered droplets were present in the liver cells of the normal rats (figures 7 and 8). In some of the anemic livers the entire cytoplasm was a fat deposit and the nucleus only did not blacken (figure 9). This tissue differed from adipose tissue in that the nucleus was not displaced by the fat deposit.

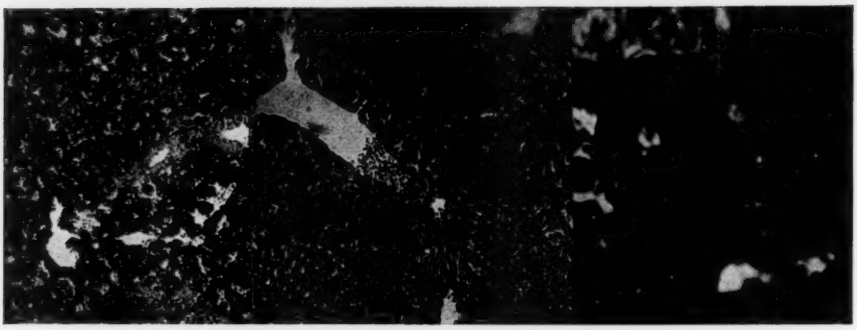
Bone marrow studies. The femora of anemic and normal littermate rats were sectioned longitudinally and stained with hematoxylin and eosin and with Mallory's stain. The bone marrow of the anemic young showed a deficiency of erythropoietic (germ) centers, a much larger proportion of mature red blood cells, and a general looseness (figures 10, 11, 12, 13, 14, and 15). This general looseness in appearance may be due to either the lack of germ centers or to fatty deposits in the bone marrow which were dissolved out by the dehydrating agents. The deficiency of germ centers was a quantitative one in that there was not a complete lack of germ centers but decidedly fewer than in the normal. Observations on the larger proportion of red cells lead us to conclude that there was not an absolute increase in the number of red cells in the bone marrow of the anemic young, but that the higher proportion was due to the marked deficiency of the erythropoietic tissue. If there were an actual increase in number of red cells, it would indicate that there is a failure of the red cells to escape from the place of origin to the blood stream. The proper interpretation, however, seems that there are fewer red cells being produced as a result of the deficiency of germ centers and that the large number of red cells present is a relative rather than absolute difference.

Other tissues. Studies of the spleen revealed no difference between the anemic and normal rat except that more blood cells were present in the spleens of the former. With the exception of the fatty deposits in the liver cells and free iron in the von Küpffer cells of the anemic rats, no histological differences in livers could be detected.

BIOCHEMICAL FINDINGS

1. Bilirubin determinations on blood

The pronounced yellowness (icterus) of the anemic rats led us to suspect a bilirubinemia. A slight modification (ELTON 1931) of the van den Bergh



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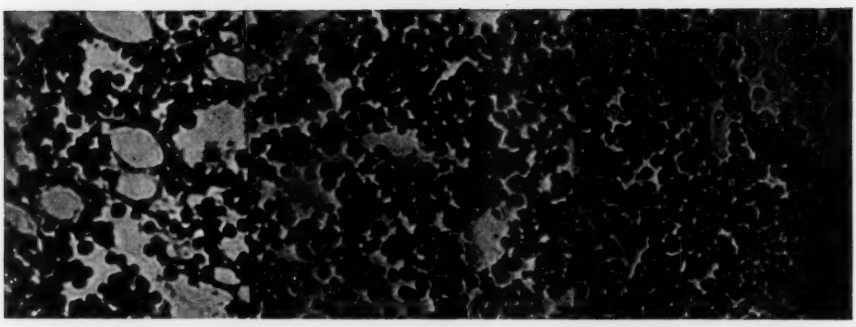

10 11 12

13 14 15

FIGURES 7–15. (See opposite page for description.)

method was used for determining the bilirubin of the blood. The earlier workers thought that the immediate appearance of the pink color at the interface of the plasma and diazo reagent (direct positive reaction) was due to bilirubinemia resulting from an obstruction to the biliary passage. If the pink color is delayed for 3–4 minutes (direct delayed reaction), it was considered an indication of bilirubinemia resulting from hemolysis, but if the pink color was delayed for 10 minutes (direct negative) it indicated a combination of the two types just described. Later work has shown that the type of reaction is dependent, not on the origin, but on the concentration of the bilirubin in the serum, since the rate of reaction is directly correlated with the icterus index (HAWK and BERGEIM 1937). Ten anemic rats 14 to 29 days old each gave a "direct positive" reaction for bilirubin in the blood plasma, whereas the same number of normal sibs gave no detectable response for bilirubin. Because of the small amount of blood available from rats of 6–9 days of age, no attempt was made to obtain quantitative values for bilirubin. The qualitative results, however, have been quite clear cut.

2. Blood uric acid

Inasmuch as there is an increased uric acid output in the urine as a result of rapid blood regeneration in which large numbers of nuclei, with their nucleic acids, are catabolized when the normoblasts mature into erythrocytes (RIDDLE 1930; KRAFKA 1929), the determination of blood

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EXPLANATION OF FIGURES 7–15

FIGURE 7.—Section of liver from a 7-day old anemic rat. Osmic acid fixation. Note the enormous fat deposition.

FIGURE 8.—Section of liver from a 7-day old normal rat. Osmic acid fixation. Only a few scattered fat deposits are present.

FIGURE 9.—Same as figure 7, but highly magnified. The cytoplasm of some liver cells is entirely a fat deposit, the nucleus being the only part of the cell which is not blackened by the osmic acid.

FIGURE 10.—Bone marrow of a 3-day old anemic rat. Note the deficiency of erythropoietic centers and the general open appearance.

FIGURE 11.—Bone marrow of a 3-day old normal rat. Note the abundance of erythropoietic tissue.

FIGURE 12.—Bone marrow of a 9-day old anemic rat. Note the deficiency of erythropoietic tissues and a larger proportion of mature red cells.

FIGURE 13.—Bone marrow of a 9-day old normal rat. Note a greater abundance of erythropoietic tissue and a smaller proportion of mature red cells.

FIGURE 14.—Bone marrow of a 3-day old anemic rat. A marked deficiency of erythrogenic tissue and a general looseness is present.

FIGURE 15.—Bone marrow of a 3-day old normal rat. An abundance of erythrogenic tissue is present.

uric acid might throw some light on the nature of this anemia. Blood uric acid was determined by the direct method of FOLIN (1922) but was proportionately modified to accommodate .2 ml of blood. The blood uric acid of the anemic (1.49 ± .03 mgm per 100 ml) did not differ significantly from that of the normal (1.47 ± .04).

3. Liver fat deposition

Histological studies on the liver of the affected rats showed a high fat content within the liver cells. The chemical findings (table 12) substantiate the histological observations in that the fat content of the anemic rat livers is 2.6 times that of the normals when expressed on a dry weight basis. Even on a "per rat" basis it is 2.7 times higher in the anemic. The liver fat was analyzed from the tissue dried to constant weight at 70°C, 20 mm Hg of pressure and in an atmosphere of carbon dioxide. These dried liver samples, consisting of composites of several rats, were then ground in a mortar and pestle and extracted with 50 ml of anhydrous ethyl ether for 24 hours. The ether was evaporated off on a steam bath; the samples were dried to constant weight in a vacuum at 70°C and weighed.

TABLE 12

Liver fat of anemic and normal rats.

GROUP	AGE IN DAYS	COMPOSITE OF	GMS FAT PER GM DRY LIVER	% FAT PER GM DRY LIVER
Normal	7	10	0.0875	11.33
	5–7	10	0.1391	
Anemic	5–7	13	0.3008	30.08

The fatty infiltration of the anemic rat livers is probably a secondary reaction brought about by a decreased oxidation in the tissues because of the lack of hemoglobin and, thus, the oxygen that it would provide. The high fat content in the livers of some anemic men is similarly interpreted (MACCALLUM 1936) but in the case of hemolytic anemias the fat may be a storage of the excessive lipids obtained as a by-product of red cell destruction.

4. Liver glycogen

Glycogen in the livers was determined essentially according to the method of PFLÜGER (DUEL et al 1934), except that the liver samples were digested for one-half hour instead of three hours in the potassium hydroxide. The glucose obtained upon hydrolysis of the glycogen was determined by the method of SHAFFER, HARTMAN and SOMOGYI, using the reagent 2 given in PETERS and VAN SLYKE (1932).

The livers of the anemic rats contain only about one-third the glycogen of the normal rat livers (table 13). The odds (Student's "t") are more than 25:1 against the view that the difference is due to chance alone. The lower glycogen content of the anemic rat liver is probably another change which is secondary to the primary anemia, although data on this point are lacking.

TABLE 13

Liver glycogen of normal and anemic rats.

GROUP	AGE IN DAYS	COMPOSITE OF	MGM GLYCOGEN PER GM FRESH LIVER	AVERAGE
Normal	5–9	3	17.4	21.0±3.7
		3	24.8	
		3	12.6	
		3	29.2	
Anemia	5–9	3	9.3	8.0±1.1
		3	5.8	
		3	8.9	

DISCUSSION

The genetics of this lethal anemia is clearly indicated, but much further work is required to analyze completely its etiology.

The results of the genetic studies show that the anemic condition is inherited as a simple autosomal recessive. No evidence has been obtained for an embryonic mortality of the homozygous recessive young because the observed ratio of 2.98:1 from 713 offspring fits the expected ratio of 3:1 very closely. The heterozygotes have shown none of the characteristic symptoms of the anemics, which indicates that the normal state is completely dominant to the anemic one. Such a physiological character which is simply inherited is very interesting and worthy of detailed study, inasmuch as most physiological characters are controlled by the more complicated multiple factors.

The possibilities of a nutritional deficiency or of an infectious cause of the anemia are eliminated by the fact that an (*An an*) female produces anemic or non-anemic offspring, depending entirely on whether she is mated to an (*An an*) or an (*An An*) male; and by the fact that with the proper parents (both heterozygous), 25 percent of the offspring are anemic and 75 percent are non-anemic although all offspring had the same "intra and extra-uterine" environment. There might be postulated a hereditary diathesis of the affected young to an invasion of micro-organisms or to an abnormal nutritional requirement for hemoglobin formation, but we have found no evidence for such an hypothesis.

Classification of this anemia in light of the facts known about human

anemias has been very difficult because its course does not parallel that of any of the common human anemias. It may well be that the difficulty resides in the fact that the anemic rats really possess two anemias: (1) the nutritional anemia resulting from the iron-poor milk diet; and (2) the hereditary anemia. It is obvious that to separate the courses of these two is indeed a perplexing task. The combined effects of the two anemias would explain the quick deaths of the affected rats.

Using a simple morphological classification, this anemia is a simple microcytic one. It is simple because the hemoglobin per red cell equals that of the normals; and it is microcytic because of the presence of small cells which decrease the mean red cell diameter. The microcytes of the anemics while decreasing the mean red cell diameter are not as concave as the normal cells; that is, they are spherocytes, and thus possess about the same volume of stroma per red cell as do the normals, and this allows them to carry as much hemoglobin.

Difficulty of classification is encountered when an attempt is made to determine whether the anemia results from faulty formation of erythrocytes (dyshemopoietic anemia) or to an excessive destruction of the red cells (hemolytic anemia). This anemia does not clearly fit into either one of the above classes. However, the present knowledge permits a tentative classification. Taking the picture as a whole, the anemia appears to result from a dyshemopoiesis. The results at hand do not contradict the idea that a faulty bone marrow is generating cells of poor resistance which are easily and prematurely destroyed; that is, dyshemopoiesis and hemolysis are both present.

The theory of dyshemopoiesis is supported by the following facts. The anemic rats possess much fewer reticulocytes than the normals, which means that in spite of the great demand for erythrocytes the erythropoietic organs (bone marrow and spleen) are unable to respond with the immature cells—the reticulocytes. Sections of the anemic bone marrows show that they possess fewer erythrogenic centers than the normals; and a cytological study of the bone marrow smears[4] of anemic young have shown a type of regeneration that is normal qualitatively but deficient quantitatively. The latter two facts can be summed up by saying that the bone marrow is hypoplastic. The dyshemopoiesis is not caused by a complete aplasia of the hemopoietic organs, for signs of some regeneration are present. The polychromophiles and microcytes are taken as evidence of a simple regeneration, whereas the multiple Howell-Jolly bodies are said to result from a pathological regeneration (DOWNEY 1930). The dyshemopoiesis involves the erythrogenic line of cells but not the leucogenic lines

[4] Kindly done by DR. H. R. SEIDENSTEIN.

because the leucocyte counts are not only equal to the normals but are higher.

Bilirubinemia and hemosiderosis are usually taken to indicate an excessive red cell destruction in which hemoglobin is catabolized into bilirubin and hemosiderin too rapidly for the body to eliminate them. This may be the case or it may be that an accumulation of such products results from an inability of the body to reuse them to form new hemoglobin. WITTS (1932) feels that the latter is the case in pernicious anemia of man.

The relationship of polychromophiles to reticulocytes has aroused much discussion. Some workers (TODD and SANFORD 1928) believe that the two are identical and that the first form is found in blood smears stained by Wright's, Giemsa's or the alkaline methylene blue methods, whereas the reticulated form is found when blood is supravitally stained. If this be true, then a contradiction seems to appear in this study where anemic blood shows much polychromasia but has a low reticulocyte count; but in the case of young rat blood, as here studied, the reticulocytes cannot be the polychromophiles, for the normal suckling rat has a very high reticulocyte count and yet blood smears do not show a correspondingly high polychromasia.

This anemia interpreted as a dyshemopoiesis allows an explanation for the time of appearance of the anemia; that is, at two to three days of life. At the time of birth in the normal rat the bone marrow takes over the hemopoietic function which heretofore has been relegated to the liver and spleen. At this time the first erythroblasts appear in the femur which already has been actively producing leucocytes (MAXIMOW 1927). With this in mind it would seem likely that the bone marrow of the anemics is unable to assume fully its function of producing erythrocytes from birth onwards, and the failure is reflected in a sudden appearance of an anemia which runs a quick and lethal course.

ACKNOWLEDGMENTS

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SUMMARY

1. A lethal anemia of rats is described which acts as a simple autosomal recessive.

2. The anemia appears spontaneously at two to four days after birth and causes death usually within two weeks.

3. The blood of anemic young possesses about one-third the number of red blood cells of the normals and about one-third the amount of hemo-

globin. In addition, there is an increased number of leucocytes and a subnormal number of reticulocytes.

4. Anemic blood smears show a marked anisocytosis, slight poikilocytosis, polychromasia and Howell-Jolly bodies. The mean red cell diameter is significantly decreased.

5. Chemical analyses have shown a bilirubinemia, fatty infiltration of the livers and a lowered amount of glycogen in the livers of anemic rats.

6. Histological studies have shown the anemic rats to have a hemosiderosis of the livers and spleens, fatty infiltration of the livers and bone marrows and a subnormal quantity of erythrogenic centers in the bone marrows.

7. The anemia appears to result from an inability of the bone marrow to assume fully its hemopoietic function at the time of birth. Accompanying this dyshemopoiesis may be some hemolysis of defectively formed red cells.

8. The anemia has not responded favorably when liver extracts, iron and copper solution, or normal rat blood have been fed or injected.

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INVESTIGATIONS ON THE PROBLEM OF METAMORPHOSIS. V. SOME FACTORS DETERMINING THE FACET NUMBER IN THE DROSOPHILA MUTANT BAR

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INTRODUCTION

IN PERFORMING eye transplantations in Drosophila it was observed that young eye discs, when transplanted into older hosts, developed into eyes containing a smaller number of facets than is normal. In order to obtain more definite information about this fact, experiments performed especially to determine some of the factors involved in the determination of the facet number in a Drosophila eye were carried out and will be discussed in this paper.

MATERIAL AND METHOD

The experiments were performed with an inbred Drosophila Bar stock. Bar was used for two reasons: (1) the reduced number of facets in Bar made facet counts easier, and (2) because Bar has often been investigated for facet number. This enabled us to compare our results with those of other investigators. The usual culture methods for Drosophila were employed. In obtaining timed larvae for the experiments, hatching larvae were selected over a two-hour range. Therefore all data concerning the age of the larvae are reckoned from the time of hatching. All experimental animals were kept at a constant temperature of 25°C $\pm$0.2. Eyes the facets of which were to be counted were fixed in alcohol, cleared in KOH and after staining with acid fuchsin were mounted in balsam. Eyes prepared in this way were drawn under the microscope with a camera lucida and facets counted in the drawing. Special details for the handling of material will be found in the text.

EXPERIMENTAL

The experiments to be described consist in transplanting young eye discs into older hosts. No distinction was made between male and female, the larvae used for the operation were selected at random. We shall analyze three experimental series in which eye discs of different ages were transplanted into hosts of the same age.

Before discussing the experiments it will be necessary to say a few words about the number of facets in the Bar stock used. It is known that the number of facets is different in the eyes of the male and the female. As a

rule the males have larger eyes than the females. The mean for the facets in the eyes of the males in our stock was 82.86 ± 2.83, and for the females 70.66 ± 1.99 (table 1). These numbers are only of value for cultures which

TABLE 1

		TIBIA LENGTH	FACET NO. ♀	FACET NO. ♂
normal	n	10	15	15
	M	67.10±0.59	70.66±1.99	82.86± 2.83
	σ	1.89±0.42	7.72±1.41	10.93± 2.00
starved to shorten larval life	n	30	8	9
	M	52.70±0.37	50.37±2.61	62.44± 4.58
	σ	2.07±0.26	7.40±1.84	13.73± 3.24
starved to prolong larval life	n	35	17	14
	M	55.17±0.82	69.00±4.14	139.71±15.25
	σ	4.89±0.58	17.04±2.93	57.09±10.78

have been well fed. In cultures with submaximal feeding the mean values are mostly smaller. Another factor in determining the facet number is, as we know, temperature. Bearing this in mind, it is obvious that the above-mentioned mean values are only relative. However, these mean values may be considered significant in our case, as in the following experiments both temperature and food were held constant to the best of our ability.

In the first instance we will discuss a series which may serve as a control. Here we transplanted 90-hour old eye discs into hosts which were ready to pupate in about six hours; thus they were more or less in the same stage as the donors. Figure 1 shows the facet number of the transplanted eyes resulting from these experiments; the mean value of the facet numbers is 69.42 ± 4.0. The experiment shows that the transplanted eyes have retained their normal size in spite of their heterotopic position.

In the next series 74–76-hour old eye discs were transplanted into hosts ready to pupate within six hours. Again the facet number of the transplanted eyes is given in figure 1. It is apparent that the transplanted eyes are somewhat smaller in size. The mean of the facet number in this series is 58.33 ± 7.49. A great variability in the size of the eye is also noticeable. Eyes with a normal facet number can occur, but on the other hand we find eyes with only 36 facets, a number never observed in normal eyes. This experiment shows that the facet number can be reduced by the transplantation of young eye discs into older hosts. It might be expected from these results that the facet number might be reduced still further by the transplantation of even younger eye discs. In order to test this, 52–54-

hour old discs were transplanted into hosts ready to pupate in about six hours. The results of the experiments may be observed in figures 1 and 2. As was expected, the facet number was still more reduced, having a mean

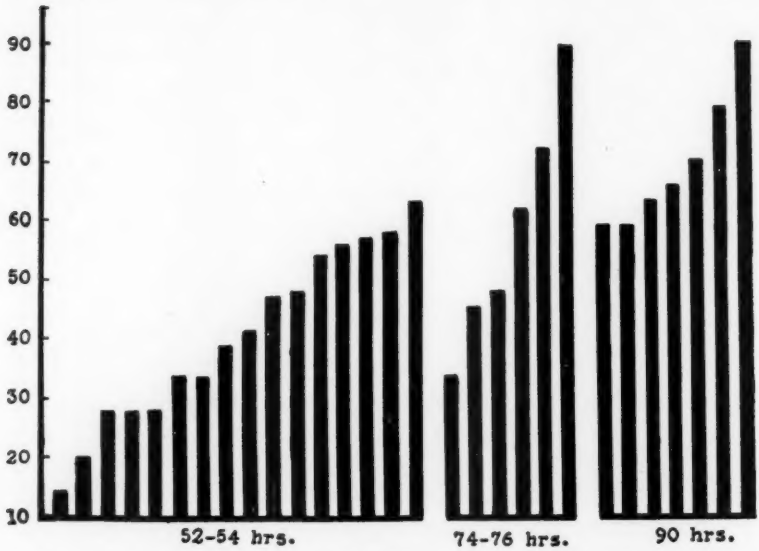

FIGURE 1.—Schematic representation of the facet number in eyes obtained by transplanting discs of various ages into older hosts.
Vertical scale: facet number.
Horizontal figures: age of transplanted eye discs.

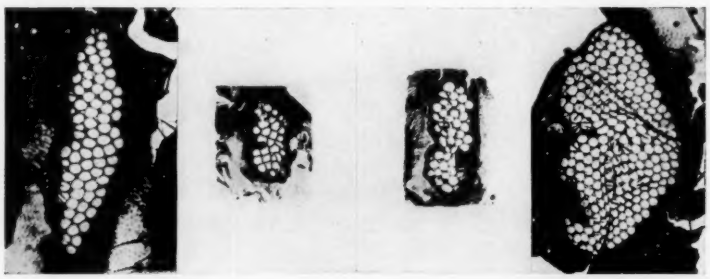

FIGURE 2.—a. Normal Bar eye.
b, c. Bar eyes obtained by transplanting young eye discs into older hosts. Note reduced facet number.
d. Bar eye obtained by prolonging larval life by means of starvation. Note increased facet number.

of 40.56 ± 3.59. Here again the different cases vary considerably; however, the number of cases with a low facet number are predominant. The smallest of this series is one having only 14 facets. This experiment shows clearly that the size of the eye can be altered by transplantation of young eye discs into older hosts. The younger the eye at the time of transplantation the fewer facets is it able to form.

It might be thought that even younger eye discs could be used in order to obtain still smaller eyes. But eye discs 30 hours of age transplanted into old hosts are not able to complete their development. Freshly hatched imagines containing such young transplants show in histological examination only partly developed eyes. These facts are in agreement with earlier experiments performed by the author (BODENSTEIN 1939a).

In order to avoid misunderstandings it should be pointed out that reduction of eye size is due to a decrease in facet number and is not a result of size reduction of each single facet. Therefore, throughout this paper if we speak of size reduction, we always mean decrease of facet number.

PAIRED TRANSPLANTATIONS

The preceding experiments have shown that young eye discs in older hosts develop fewer facets than normally. Apparently, at the time of pupation the formation of new facets ceases, whereas during pupal life only the differentiation of previously formed elements takes place. From this one might suppose that in our experiments the differentiation processes in the young eye discs take place sooner than is normal, even before the discs have reached their normal facet number. Hence it might be thought that the length of time at the disposal of the eye discs in the larvae could be decisive for the final number of facets. If so, equivalent eye discs transplanted into an old and a young host should develop fewer facets in the first case than in the second. The following experiment was therefore performed.

The two eyes of single 52-hour old donor larva were transplanted, one into an old host and the other into a young host. Since in such a pair of hosts the transplanted eyes came from the same donor, one is certain to have transplanted eyes of the same age. Seven of such pairs are at our disposal. Figure 3 shows the results of these experiments. It is very evident in all pairs that those eyes transplanted into older hosts (a) are considerably smaller than the partner eyes which have been transplanted into the younger hosts (b). In other words, eye discs having a longer time from the operation to pupation at their disposal have developed larger eyes than those undergoing pupation shortly after the operation. This fact also becomes clear when the experimental series is arranged according to the number of days the transplant has been in the larva, as is shown in table 2.

The mean of the facet number for transplanted eyes, the hosts of which pupated 0 days (soon after operation), one day and two days following transplantation is 33, 40 and 89.8, respectively. The experiments so far performed show that by the transplantation of eye discs of different ages into older hosts of the same age, or, vice versa, by the transplantation of discs of the same age into hosts of different ages, it is possible to vary the

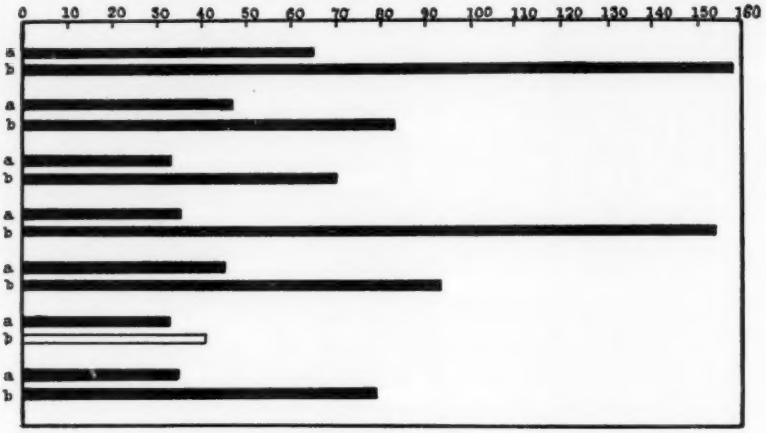

FIGURE 3.—Schematic representation of the facet number in eyes obtained by transplanting eye discs of a single donor into hosts of different ages.
a. Facet number of transplanted eye in older host.
b. Facet number of transplanted partner eye in younger host.
Horizontal scale: facet number.
Eye represented by white block is incomplete.

facet number of the transplant at will. The length of time the eye disc remains in the larva, or, in other words, the time of onset of differentiation, determines the number of facets.

TABLE 2

Transplantation of 51–53-hour old eye discs into hosts of different ages; showing relation between number of facets and the time the disc remained in the larva after transplantation.

DAYS TRPL. EYE DISC REMAINED IN LARVA	NUMBER OF FACETS						M.
0	33	33					33.0
1	35	35	45	47			40.5
2	60	70	79	83	93	154	89.8

STARVATION EXPERIMENTS

Since we have shown that the number of facets depends upon the length of time the disc remains in the larva, it was of interest to determine whether an experimentally produced change in the length of larval development might also effect a change in the number of facets. According to this point of view one should expect that in shortening larval life the number of facets will decrease, whereas by lengthening larval life an increase of facets should occur. It is known among entomologists that starvation may accelerate or delay pupation. In connection with studies on eye color substances in Drosophila, BEADLE, TATUM, CLANCY (1938) have recently performed starvation experiments in Drosophila. These investigations now prove to be very useful to us. They found that there is a critical period in larval life after which total starvation causes a somewhat accelerated pupation. This critical period occurs in the larva at about 70 hours (from oviposition). On the other hand, before this time a low food level causes prolonged larval life, that is, a great delay of pupation.

From the above consideration, it becomes clear that larval life may be increased or decreased by starvation. Two sets of experiments were then performed which might shed some light on our problem. In order to shorten larval life, 57-hour old larvae were washed and starved completely in a dish containing only moist filter paper. These animals pupated several hours earlier than they would have done without starvation. Table 1 shows the result of this experiment. It is evident that the numbers of facets in both male and female are clearly reduced. A comparison of the mean values of the facet numbers in the normal series shows that the difference is significant. All of the starved animals are very small, as is indicated by the measurements of the tibia lengths, which, according to BREHME (1939) is a good criterion for the size of a fly. The standard deviation in the tibia measurements shows the constant size of the flies. This experiment again seems in favor of the fact that the length of time the eye remains in the larva is decisive for its final size.

In order to prolong larval life freshly hatched larvae, as well as one- and two-day old larvae were put on a low food level. The low food level medium consisted of a 1.5 percent agar suspension, containing 0.25 grams air dried brewers' yeast. The larvae did not withstand the starvation very well, especially those starved directly after hatching. The time of development of larvae starved in this way varies considerably. Some flies hatched with a delay of only one day, whereas others were delayed up to five days. Since it was found to be immaterial for the time of delay whether the larvae were starved shortly after hatching from the egg or whether they were starved at an age of two days, the three series were treated as one. Table 1 shows the results of these experiments. We observe here that the

flies are smaller than normal, as is indicated by the mean of their tibia lengths. It is also noticeable that the standard deviation of the tibia measurements is relatively large, showing a greater variability in size than was found in the foregoing experiment. The mean facet number of the females is not very much changed, but the standard deviation is greater than in the normal series. This suggests a probable influence of starvation. A much clearer effect of starvation may be observed in the males of the series. Here the mean value for the facet number is increased to 139 as

TABLE 3

Relation between delay of emergence and facet number.

FEMALE		MALE	
DELAY IN DAYS	NUMBER OF FACETS	DELAY IN DAYS	NUMBER OF FACETS
1	77	2	215
1	89	2	193
2	77	3	259
2	90	3	211
3	112	3	157
3	76	3	140
3	75	3	130
3	73	4	108
3	70	4	98
3	69	4	87
3	62	4	87
3	49	4	70
4	58	5	94
4	58	5	93
4	57		
4	42		
5	44		

compared with a mean of 83 in the normal series. Again we find a high standard deviation. In this series some of the males had eyes with 260 facets, a number much above the highest number observed in a normal series. Comparison of the mean facet number in the males of this series with that of the normal series shows that the difference is significant.

In summarizing the results of these experiments we find that by the starvation method used it was possible to prolong larval life, with an accompanying increase in the number of facets. These results support our above mentioned point of view that the length of time the disc remains in the larva is important for its final size.

As the following considerations will show, however, the problem in question is not so simple. We have already mentioned that the time of development of the larva varies considerably in the experiment. If time

of development were the only factor determining the final number of facets, one would expect those larvae having a long developmental period to have larger eyes than larvae having a shorter one. This is not the case. In fact, the opposite seems to be true. In table 3 the eye facet number (male and female) of each is compared with the time of delay. Animals delayed five days may have an approximately normal number of facets, whereas those animals delayed one or two days may have a considerably increased number of facets. The distribution of the cases gives the impression that a decrease of larval lifetime brings about an increase in the number of facets. The number of cases is, however, hardly sufficient to establish this fact. Further experiments will be necessary. But in any case, it is certain that a long larval period does not necessarily bring about an increase of facet number. The fact remains, however, that the mean value of the facet number in the experiment under discussion is higher than normal. Thus we may conclude with reservations that, as expected, prolonged larval life actually causes an increase in facet number.

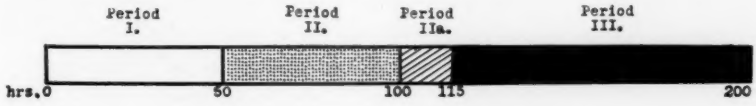

FIGURE 4.—Diagram showing physiological periods in the development of the eye.

DISCUSSION

The development of the eye

In an effort to elucidate the problem in hand, a diagram (figure 4) illustrating the development of an eye disc was constructed according to the experimental data. After the larvae have hatched, the eye discs grow very rapidly up to 50 hours. This developmental period (0–50 hours) is represented by period I in the diagram. Whether the physiological condition of the eye disc changes in this time is so far uncertain. A physiological change in the eye disc must occur at about 50 hours, because eye discs taken from larvae before this time and transplanted into older hosts (see page 497 and BODENSTEIN 1939a) are not able to differentiate normally, whereas eye discs taken from larvae after 50 hours and transplanted into the same host differentiate normally. At the time of 50 hours, therefore, a fundamental change takes place in the eye discs. This change at 50 hours, demonstrated by the transplantation experiments, corresponds to the time at which (MEDVEDEV 1935) the eye disc separates from the antennal buds, and is now clearly visible as an imaginal eye disc. The eye disc at this time is morphologically and histologically very much like an eye disc of an old larva, only smaller. From this one might expect that

after 50 hours only growth occurs and the physiological condition remains unchanged. In fact, ENZMANN and HASKINS (1938) studying the development of the eye disc in Drosophila came to the conclusion that during this period (period II) only growth took place. They found further that the growth processes consist in cell division, whereas the size of each cell remains the same. Our transplantation experiments show in addition that the eye disc does not change physiologically in period II. This conclusion is based upon the fact that the eye discs throughout period II behave alike in the transplantation experiment. There is, however, a striking difference between younger and older eye discs, namely, younger eye discs form fewer facets than the older discs in the experiment. These results also indicate that only cell division takes place in period II.

Experiments of BEADLE and EPHRUSSI (1936) are also instructive in this connection. These authors found that young eye transplants of *melanogaster* (wild type) into older hosts gave rise to eyes markedly smaller than those resulting from transplantations where there was no age difference between transplant and host. As preliminary experiments indicate, the typical behavior of the young Bar eye transplants is not a peculiarity of this species, for we also obtain eyes with a reduced facet number when young eye discs of *D. melanogaster* (Oregon R) or *D. hydei* are transplanted into older hosts. It is thus reasonable to believe that the small eyes observed by BEADLE and EPHRUSSI in their experiments are also due to a reduction in facet number.

It is evident from the work of MEDVEDEV and ENZMANN-HASKINS that the growth of an eye disc decreases constantly with the age of the larva, finally ceasing completely at pupation. The question may now be raised whether the gradual decrease of the growth rate until cessation at the time of pupation is an expression of an inherent tendency in the eye disc itself or whether factors in the organic environment of the eye, that is, the physiological condition of the larvae at the time of pupation, are responsible. Our experiments shed some light on this question. Since we find that young eye discs transplanted into older hosts form fewer facets, we may conclude that the host must have determined the time of cessation of growth at pupation. There is still the possibility to be considered that some processes of growth of the young transplanted eye disc may occur in the pupa. This, however, is improbable, since at emergence of the host the transplanted young eye discs have reached the developmental stage of the host eyes. It is also known (BODENSTEIN 1939) that the rate of differentiation cannot be altered. The typical result of the experiment, that is, smaller eyes, must have been caused by an earlier cessation of growth in the larva.

We have shown that the eye discs in 50-hour old larvae have reached

the physiological condition of full grown larvae. Furthermore, this time corresponds to the separation of the eye disc from the antennal bud, and to the completion of the histological structure of the larval eye. The following facts are of further interest: in starvation experiments BEADLE, TATUM and CLANCY (1938) found that larvae 70 hours of age could be starved completely, yet could still pupate, while larvae starved before this time were incapable of doing so. These 70-hour old animals, completely starved, do not increase in size. Since these animals are able to pupate in spite of complete cessation of growth, the larvae must by this time have reached a developmental stage in which their organs are reactive to the pupation factors. In the starvation experiments the larvae reach this stage, as we have seen, at 70 hours. Since BEADLE and his collaborators measured age from oviposition, this 70-hour period corresponds with our 50-hour period measured from the time of hatching from the egg. At this time in development the eye discs reach a stage equivalent to that of a full grown disc and are thus capable of the changes normally involved in pupation. If one assumes in this connection that no further growth occurs in starved larvae before pupation, animals starved completely at 57 hours should have the same sized eyes at pupation as they had at the beginning of starvation. By starvation, therefore, those growth processes normally taking place in period II from 57 hours until pupation are excluded. The same can be achieved by transplanting 55-hour old eye discs into larvae about ready to pupate. In both cases, that is, by complete starvation and by transplantation the same period of growth is eliminated. The eye discs should thus have finally the same size. We find, however, that the eyes of the starved animals are much larger than those eyes resulting from the transplantation experiments. This fact indicates that, although the starved larvae did not increase in size, their eye discs must have continued to grow, showing a selective effect of starvation on growth. We will refer to this fact again later.

Returning to our diagram of development we find that the beginning period IIa is characterized by the onset of pupation; at this time, the growth of the disc ceases. It is most likely that during pupation certain changes occur in the physiological condition of the eye disc, the nature of which are so far still uncertain. We know only that at about 25 hours after pupation the imaginal disc begins with its differentiation processes. Once in progress, these processes continue at a rate which is species-specific and which cannot be changed. (See BODENSTEIN 1939a.) From the actual beginning of pupation, that is, from the evagination of the imaginal discs until the onset of differentiation the disc seems to remain at a latent stage. The onset of this differentiation period can be changed experimentally, thus it is possible to shorten or prolong the latent period (BODEN-

STEIN 1939b). It is, however, still uncertain how far this period can be shortened or lengthened. Presumably we can exclude the latent period altogether. On the other hand, the latent period may be extended as long as the animal has reserves which provide energy supporting life-processes. This length of time is, of course, dependent upon temperature, which largely determines metabolic processes. In the diagram (figure 4) period IIa includes firstly the time of prepupation, which ends with the evagination of the imaginal discs, and secondly, the latent period. Probably the changes in the physiological condition of the disc which give the necessary provision for processes leading up to imaginal differentiation occur within this prepupation period. It is not possible in this connection to go into further detail concerning period IIa; this will be discussed in another publication.

The last physiological stage is represented by period III in our diagram. This is the actual period of differentiation. Here the disc is brought to its imaginal completion (BODENSTEIN 1939a).

The determination of the eye disc

Some information concerning the determination of an eye disc may also be obtained from our experiments. As we have seen, the eye disc at the age of 50 hours is completely formed. It consists, however, of relatively few cells. The disc acquires its final size by growth in period II. The question may be raised whether the 50-hour old eye anlage is at this time already a determined mosaic or whether the eye disc is still capable of regulative processes, which for example render possible the regulation of half an eye disc to normal size within period II. The question might also be stated as follows: are the growth processes of half an anlage different from the processes of growth of a complete anlage in period II. Thus the problem becomes one of growth regulation. Experiments performed especially to elucidate this question are not available; only some accidental observations are at our disposal, which may be explanatory here. It was found that eye discs torn in the process of transplantation gave rise to small eye fragments, each consisting of a certain number of facets. The total number of these facets in the fragments corresponded approximately to the number of facets in a normal-sized eye, indicating that no appreciable regulative processes had occurred. Eye discs of approximately 50-hour old larvae may thus be considered as determined. These additional observations find their confirmation in the work of HASKINS and ENZMANN (1937). These authors found that when young larvae were treated with X-rays, stronger eye disturbances were produced than when older larvae were subjected to the same dosage. This may mean: if in a young eye disc two cells each belonging to a cell-group forming later an ommatidia, are

killed by X-ray particles, the injured cell-group will divide three times before pupation, thus giving rise to sixteen ommatidia, having a definite defect. If, however, two cells of an older eye are hit, the injured cell-group will divide once only before pupation, resulting in only four injured ommatidia. With regard to our problem this suggests that at the time of X-raying, each element in the cell-group was clearly determined as to its final destination; no regulation had taken place.

We may summarize the foregoing in stating that all elements indispensable for the structure of the imaginal eye are determined quite early in the development of the eye disc. Once determined the anlage is only capable of growth. It should, however, be pointed out that the word determination is used in a very special sense.

Systems involved in the determination of the facet number

In the following we do not attempt to give a final interpretation of the facet problem. We wanted to emphasize rather a special angle of this problem, since we believe that some of the later mentioned considerations will be useful for further investigations.

In transplantation and starvation experiments already discussed, a correlation was observed between the number of facets and the length of larval life. Since other factors too have been found capable of changing the facet number in Drosophila some of these may be discussed briefly in this connection. In temperature experiments, for instance, it was found that generally the facet number and larval life were inversely proportional to temperature (KRAFKA 1920, LUCE 1926, HERSH 1930 and his collaborators). By oxygen treatment, which may also alter the facet number, we find again a correlation between length of larval life and facet number (LUCE 1938). On the other hand, there are a number of investigations where no relationship between larval life and number of facets was observed. Our starvation experiments, where a very much prolonged larval life is not necessarily followed by an increase of facet number might serve as an example. Also MARGOLIS (1938) makes no mention of any such relationship in his oxygen experiments.

Naturally the question occurs, can we arrive at a general explanation of the problem, in spite of these discrepancies? As the developmental processes are so highly complicated, we will endeavor to simplify our explanation as much as possible. Two postulates might be made which are based on the experimental data: (1) The eye disc possesses a definite rate of growth. (2) The growth rate depends on the physiological condition of the larvae at any given time.

The factors influencing the facet number may thus act upon the growth rate of the eye anlage itself; they may influence the physiological con-

dition of the surrounding tissue environment or both simultaneously. In each case a change in facet number will result. In the following some experiments will be discussed in the light of these postulates.

Let us suppose firstly that the treatment affects the environment alone. This may bring about a prolongation or retardation of larval life. In the former case we should expect a large eye and in the latter a smaller one. The transplantation experiments seem to demonstrate these two possibilities since we find that the size of the eyes depends upon the length of time they remain in the larval environment. Certain temperature experiments (see above) and oxygen treatments (LUCE 1938) where a change in facet number is accompanied by a change in larval life, may indicate the same. It is, however, difficult to decide in these cases whether the treatment affects only one of the systems involved or both simultaneously. Although the results of these experiments are similar to our results, obtained by transplantation, where the physiological condition of the host is clearly responsible for the size of the eye, there is still the possibility that the anlage itself may also be affected here.

Should the treatment affect the anlage itself, a smaller eye will develop when the growth rate is inhibited, and a large eye when the growth rate is stimulated. Experiments of MARGOLIS (1939) may be mentioned in this connection. He found that Bar larvae, when treated with oxygen at an age of 24–72 hours showed a more marked increase in facet number than larvae treated from 48–90 hours. Supposing here that oxygen stimulates the growth rate of the anlage, we can understand that young eye discs are proportionally more stimulated than older ones, because in normal development the growth rate of the disc decreases constantly with age.

The third possibility is that both systems, that is, anlage and environment, may be affected simultaneously, which seems to be the case in our starvation experiments. Here we find that normal eyes can occur in spite of a very much prolonged larval life. If the environment alone is affected (inhibited) we expect according to our conception a very large eye, because when larval life is prolonged the disc has a longer time of growth at its disposal. As we find, however, a normal eye, we must conclude that both anlage and environment are affected. In this case then, the increased time for larval growth compensates for the reduced growth rate of the anlage.

Some additional comment may be made on this experiment. We can assume that the prolongation of the larval period is an indication for the strength of the effect produced by starvation. If both anlage and environment are equally influenced by starvation, it is immaterial whether the effect is strong or weak; the increased time for growth and the reduced growth rate should always bring about normal eyes. It was, however, found (see page 500) that flies retarded five days in the larval period could

have normal eyes, whereas flies retarded three days or less were found to have larger eyes than is normal. These observations, then, seem to indicate that the starvation effect has to reach a certain threshold before it can act on the anlage itself. Before this level is reached, the effect is only on the environment, thereby giving the normal eye disc a longer time to develop, resulting in a larger eye. These considerations may serve to explain the irregular results of the starvation experiments mentioned on page 501.

In the foregoing we have been concerned solely with the problem from the developmental point of view. It was pointed out that there probably exist two developmental systems which in collaboration determine the final number of facets in a Drosophila eye. We consider it quite futile at present to relate the considerations mentioned here to the genetic aspect of the problem. We might suggest, however, that in the light of our discussion, genes influencing the facet number may act upon one of these developmental systems, by breaking their relationship in the same manner as our transplantation, starvation and other experiments discussed here, have done.

SUMMARY

1. Transplantation and starvation experiments in Bar-eyed Drosophila were performed in order to elucidate some of the factors involved in the development of the eye.

2. In transplanting young eye discs into older larvae it was found possible to reduce the number of facets in the transplanted eyes. The younger the transplanted eye discs, the fewer facets developed. Eye discs younger than 50 hours (from hatching from the egg) did not, however, develop normally.

3. It was thought that the length of time the disc remained in the larva was responsible for this effect. In order to test this, the eyes of a single donor larva were transplanted, one into an older host and the other into a young host. It was found that those eyes in older hosts developed fewer facets than their partner eye in the younger hosts. It thus follows that discs remaining a long time in the larva develop more facets than discs having only a short time at their disposal for development.

4. By starving larvae completely larval life could be shortened. Flies, the larvae of which had been treated in this way, showed a slight but significant decrease in facet number.

5. The imaginal eyes of flies, the larval life of which had been prolonged by starvation, showed in some cases a great increase in the number of facets.

6. An attempt is made to give a picture of the developmental sequences in the Drosophila eye.

Some facts concerning the determination of the eye are mentioned.

It is discussed in which way certain developmental systems may be altered in order to bring about a change in facet number.

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CYTOGENETIC STUDIES WITH POLYPLOID SPECIES OF WHEAT. I. CHROMOSOMAL ABERRATIONS IN THE PROGENY OF A HAPLOID OF TRITICUM VULGARE[1]

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INTRODUCTION

THE usual methods of genetic analysis lose much of their efficiency when applied to polyploid organisms, where duplicate factors tend to mask recessive gene mutations, and where the large number of chromosomes greatly increases the difficulty of determining linkages. A different way of analyzing polyploids is being tried with *Triticum vulgare*. This method is based on the fact that whole-chromosome deficiencies and duplications are viable in *T. vulgare*, and that these aberrations, as well as reciprocal translocations, can be obtained from haploids of this polyploid species. The origin and possible uses of two reciprocal translocations and a number of whole-chromosome aberrants from a haploid of *T. vulgare* will be discussed in this paper.

PROGENY OF A HAPLOID WHEAT PLANT

Two haploids were found in a field culture of 105 plants grown from seed of *T. vulgare* var. "Chinese Spring" (n=21) pollinated by *Secale cereale* (n=7). They were not conspicuously different from diploid Chinese Spring wheat, except for complete male sterility. One of the haploids was completely female sterile, but the other set 14 seeds from less than 300 florets pollinated by normal wheat pollen, and nine seeds from a somewhat larger number of florets pollinated by rye. Successful pollinations involved several different spikes from various parts of the plant, and were made over a period of about two weeks.

From the nine seeds of the haploid pollinated by rye, two mature plants were obtained. These had 27 and 28 chromosomes, respectively, and resembled ordinary wheat-rye hybrids. Neither plant produced seeds.

The 14 seeds of the haploid pollinated by diploid wheat yielded 13 mature plants. Cytological examination at meiosis in these plants, supplemented by similar studies of their progeny, provided the information given in table 1. The aberrant chromosome numbers and pairing relationships of plants 1 to 11 presumably resulted from abnormal chromosome consti-

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tution of the eggs produced by the haploid. Although no study was made of microsporogenesis in the plants used as pollen parents, several other diploids of Chinese Spring wheat showed regular meiotic behavior. At least two different individuals were involved in the male parentage of the aberrant plants.

TABLE 1

Results of cytological analysis of the progeny of a cross, Triticum vulgare haploid×T. vulgare diploid.

DESIGNATION OF PLANT	SOMATIC NUMBER	BIVALENTS	UNIVALENTS	TRIVALENTS*	RINGS OF FOUR
1	41	20	1		
2	41	20	1		
3	41	20	1		
4	41	20	1		
5	41	20	1		
6	42	19	1	1	
7	40	19	2		
8	41	18	2	1	
9	41	18	2	1	
10	40	17	2		1
11	42	15	2	2	1
12	42	21			
13	42	21			

* Trivalents were frequently replaced by a bivalent and a univalent.

DESCRIPTION OF THE ABERRANT PLANTS AND THEIR PROGENY

Selfed seeds were obtained from all the aberrant plants but one. Some individuals were abundantly fertile under bag, while others had to be pollinated by hand. The poorly self-fertile plants, including the one which had set no seeds, were later back-pollinated successfully by normal Chinese Spring wheat.

Plants with a single univalent

Five individuals were characterized at meiosis by the presence of twenty pairs of chromosomes and one univalent.

In the following descriptions, numbers given to plants correspond to those in table 1.

1. Plant number 1 showed a slight chlorophyll defect. From 20 selfed seeds, 20 plants were obtained, of which two and possibly a third were monosomic, and two were nullosomic (20 pairs of chromosomes). The monosomic plants under field conditions were somewhat smaller and less vigorous than disomic sibs, and were lighter in color. The nullosomics were bushy dwarfs with stiff leaves more nearly normal in color than those of the monosomics. Neither of the nullosomic plants set seeds, but enough

functional pollen was obtained from one of them to produce a viable seed in a cross to *T. durum*.

2. Plant number 2, grown under greenhouse conditions, was nearly normal, differing mainly in the greater thickness and stiffness of its culms. Among its field-grown progeny, however, the only monosomic which occurred was dwarfed and sterile. The 28 plants (from 30 selfed seeds) included no nullosomics.

3. This monosomic was somewhat defective in size and vigor, although normal in color. From 20 selfed seeds, 16 plants were obtained, including three monosomics and no nullosomics. The aberrant plants resembled their parent in size and vigor.

4. This plant did not differ noticeably from normal, but in the field its monosomic offspring were somewhat lighter in color. Of 34 plants which resulted from 35 selfed seeds, three were found by cytological examination to be monosomic, and several which were not studied cytologically had the characteristic color of monosomics. No nullosomics were observed.

5. This was a rather small, weak plant, which did not set selfed seed until late in the season. Two daughter plants were obtained from four backcrossed seeds, and both were monosomic. These were more vigorous than the parent.

Plant with one univalent and one trivalent

6. No differences from the normal could be detected in plant number 6, nor could the monosomic and trisomic plants in its progeny be identified other than cytologically. From 16 selfed seeds, 16 plants resulted. Of the 15 of these which were studied cytologically, only two were normal, six were monosomic (figure 1), five had one univalent and one trivalent (figure 4), one had a univalent and a quadrivalent (figure 5), and one was nullosomic. The nullosomic plant, although small and lacking in vigor, had normal pollen fertility and set a number of selfed seeds. The monosomic-tetrasomic plant was normal, or nearly so, in appearance and fertility.

Plant with two univalents

7. This plant had narrow leaves and slender spikes and culms. From seven selfed seeds, six plants were obtained, of which five were examined cytologically. Four were of the parental constitution (figure 2), although phenotypically nearer normal, while the fifth had 20 bivalents and one univalent.

Plants with two univalents and one trivalent

8. This was the smallest and weakest of the 11 plants. It had a very short spike and shed no pollen. The only plant obtained from the three

backcrossed seeds had one univalent and one trivalent, and was much more vigorous than its parent.

9. This individual had a glossy appearance, and produced a short tapering spike with an extra spikelet at each of several central nodes. Two selfed seeds were obtained, and these produced one plant of the parental constitution (figure 3) and one with a single univalent. A single backcrossed seed gave an individual with one univalent and one trivalent.

Plant with two univalents and a ring of four

10. This plant was characterized by a dark green striping of its leaves. From 22 selfed seeds 21 plants were obtained, of which all but six were studied cytologically. Three were of the parental constitution (but failed to show the stripe), seven had one univalent and a ring of four, one had one univalent and a chain of four, three had two univalents, and one had one univalent. Figure 6 shows the appearance of the ring at first metaphase.

Plant with two univalents, two trivalents, and a ring of four

11. This plant was not conspicuously abnormal, its chief distinctive characteristic being a glossy appearance. Three plants were obtained from three selfed seeds, and two of these were examined cytologically. One, which died without setting seed, had two univalents, one trivalent, and a ring of four. The other plant had a trivalent and a chain of four. From 11 backcrossed seeds, 10 plants resulted. Two of these had two univalents and two trivalents; one had two univalents, one trivalent, and a ring of four; two had two univalents and one trivalent; one had one univalent, one trivalent, and a ring of four; one had one univalent, one trivalent, and a chain of four; two had one univalent and one trivalent; and one was normal.

EXPLANATION OF PLATE

Photographs from aceto-carmine smears, showing first metaphases (except figure 3) in aberrant plants. ×850.

FIGURE 1.—A monosome with a conspicuous, sub-terminal constriction, from an offspring of plant 6.

FIGURE 2.—Two monosomes, one considerably longer than the other, from an offspring of plant 7.

FIGURE 3.—Two monosomes at first anaphase, showing the chromatids passing to the poles. The upper chromosome has a median centromere, the lower a sub-terminal centromere. From an individual in the progeny of plant 9.

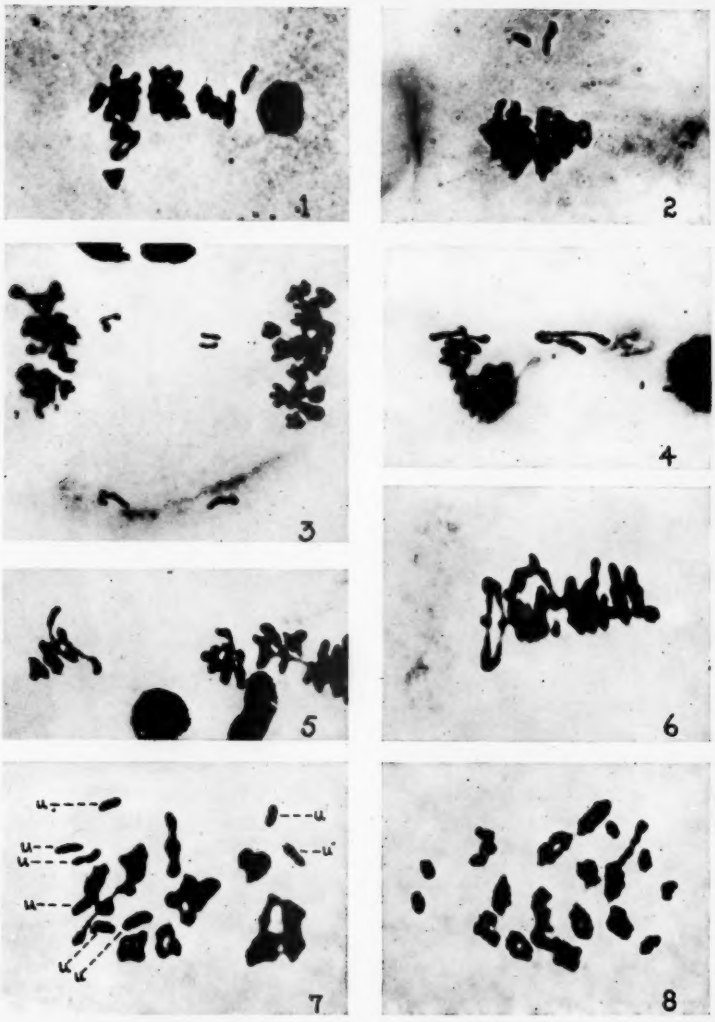
FIGURE 4.—A trisome, from an offspring of plant 6.

FIGURE 5.—A tetrasome (at left), from an offspring of plant 6.

FIGURE 6.—A ring of four, from an offspring of plant 10.

FIGURE 7.—Thirteen bivalents and eight univalents in a 34-chromosome hybrid of *T. durum* with a monosomic offspring of plant 3. u=univalent.

FIGURE 8.—Fourteen bivalents and six univalents in a 34-chromosome hybrid of *T. durum* with a monosomic offspring of plant 10.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GENERAL CONSIDERATION OF THE ABERRATIONS

Monosomes

In the chromosome constitutions of the 11 aberrant plants in the immediate progeny of the haploid, 16 monosomes were involved, of which two were lost in the succeeding generation. Where two of these occurred in the same plant, as they did in five instances, those two necessarily involved non-homologous chromosomes; and other differences between the monosomes have been indicated by differences in appearance of monosomic plants and by differences in the size and morphology of the chromosomes concerned (figures 1, 2, 3). The chance of all 14 monosomes being different is small, however, even though they may have occurred at random.

The effort involved in determining homologies amongst the 14 monosomes would be much less if there were reliable cytological means of identifying the chromosomes. Recent work of BHATIA (1938) indicates that cytological distinctions do exist among most of the chromosomes.

Since the 14 chromosomes of emmer wheats pair regularly with 14 of the 21 *vulgare* chromosomes (SAX 1922), a 34-chromosome hybrid of emmer with a monosomic plant of *T. vulgare* will show at meiosis whether or not the monosome concerned is a chromosome homologous with one of emmer. Of eight monosomes thus far tested, five (figure 7) have proved to have a homolog in the emmer complement, while three (figure 8) are among the extra seven chromosomes of *T. vulgare*. *T. durum* was the emmer wheat used.

Deficiencies of whole chromosomes or parts of chromosomes have been observed several times in 21-chromosome wheats. KIHARA (1924) obtained in the F_4 of a cross between *T. polonicum* and *T. spelta* two plants which had only 20 pairs of chromosomes and which bred true. One was a dwarf with a somewhat lowered seed set, while the other was semi-dwarf, with nearly normal fertility. NISHIYAMA (1928) studied the monosomic plants which were obtained from these two lines through crosses with normal plants. He found that such monosomics produced functioning 20-chromosome gametes, though the proportion was much lower among male than among female germ cells.

HUSKINS (1928, 1933) and HUSKINS and SPIER (1934) have shown that certain types of speltoids are due to deficiency of a whole chromosome or a part of a chromosome. Where a whole chromosome is missing in the speltoid, homozygotes (that is, nullosomics) are rarely obtained, although selfing gives 5 to 20 times as many heterozygotes as normals. Where only a part of a chromosome is missing, homozygous speltoids are more frequently obtained, giving typically ratios near 1:2:1, and sometimes approaching 1:1:1.

UCHIKAWA (1937) also has found 41 chromosomes in a heterozygous speltoid. The same chromosome number was observed in a short, but otherwise normal, plant.

LOVE (1938) showed that white-chaff off-types in a golden chaff wheat were homozygous for a deficiency of one chromosome arm. LOVE (1939) also reports in F_5 to F_7 of *vulgare-durum* crosses *vulgare*-like plants deficient for as many as four chromosomes.

KATTERMANN (1932) isolated 40- and 41-chromosome plants from an F_6 of a wheat-rye cross.

Trisomes

Not as many instances of trisomes in *T. vulgare* have been observed as of monosomes. HUSKINS (1928, 1933) believed that certain speltoids were due to the presence of an extra chromosome or pair of chromosomes. UCHIKAWA (1937) reported that a semi-*compactum* wheat had 43 chromosomes, and its short-*compactum* derivative 44 chromosomes. In *vulgare*-like derivatives of *vulgare-durum* crosses, LOVE (1939) found numerous trivalents, some of which probably represented trisomes.

Thus far, two of the five trisomes obtained from the haploid of *T. vulgare* have been separated from the monosomes which accompanied them in the original offspring of the haploid. Neither differs conspicuously from normal in phenotype. From one trisomic plant both pollen and ovules of n+1 constitution have functioned; a daughter plant of a selfed trisomic individual (number 6) was tetrasomic (figure 5).

Reciprocal translocations

The two associations of four[3] observed were presumably reciprocal-translocation configurations rather than tetrasomes. Rings of four occurred with greater regularity than would be expected for tetrasomes, which frequently form a chain of four, or two pairs. Also, since the male parent presumably contributed only one each of the 21 kinds of chromosomes, a tetrasome would necessarily have involved three homologs from the maternal parent. The occurrence of gametes from a haploid with three homologs is difficult to explain.

Additional evidence is available that the ring of four found in plant 10 (figure 6) was due to a reciprocal translocation. The 17 bivalents and two univalents account for only 19 paternal chromosomes, leaving two to be involved in the ring. Furthermore, if the association of four had been a tetrasome, the plant would have been deficient for one pair of chromosomes, in which case it would probably not have been as vigorous as it was.

[3] There is some evidence from a later generation that a third reciprocal translocation was present in the immediate progeny of the haploid (in plant 7).

The interpretation of the ring of four in plant 11 as a reciprocal-translocation association is substantiated by the pairing relationships in one of its offspring. This daughter plant (from a selfing) had 43 chromosomes, 36 of which always formed 18 pairs. The other seven were observed to form a trivalent and a chain of four; a bivalent, a univalent, and a chain

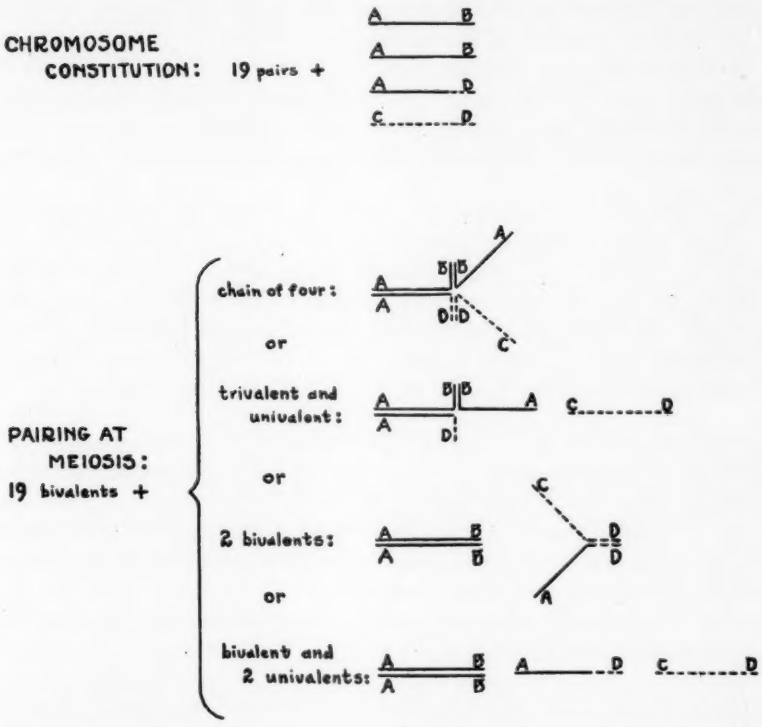

FIGURE 9.—The chromosome constitution and meiotic behavior of a plant which would occur in the progeny of an individual with a ring of four if one of the parental gametes received adjacent chromosomes from the ring and therefore had a duplication and a deficiency.

of four; two trivalents and a univalent; a trivalent, a bivalent, and two univalents; or two bivalents and three univalents. Part of this variability can be accounted for by the presence of a trisome, which sometimes formed a trivalent and sometimes a bivalent plus a univalent. The remaining four chromosomes presumably represented an unstable chain of four, which frequently broke up (figure 9) into a trivalent and a univalent, into two bivalents, or into a bivalent and two univalents. Such a chain of

four would be expected in the progeny of a plant with a reciprocal-translocation ring of four, as a result of the functioning of a duplication-deficiency gamete.

Although neither of the two reciprocal translocations has yet been obtained in a plant which was free from monosomes, it has been possible to determine that they bring about no great reduction in male or female fertility. It is yet to be learned whether this lack of sterility is due mainly to directed segregation of the members of the chromosome ring, as shown by THOMPSON and THOMPSON (1937) in *T. durum* and by these authors (1937) and by SMITH (1939) in *T. monococcum*, or mainly to the ability of deficiency-duplication gametes to survive. In the one instance just discussed the functioning of a deficiency-duplication gamete has been indicated.

A reciprocal translocation in *T. vulgare* has been discovered by KOSTOFF (1937) in a 42-chromosome segregate from a cross of *T. vulgare*×*T. monococcum*. KATTERMANN (1934, 1935a, b) backcrossed a wheat-rye hybrid to wheat and obtained plants with multivalent associations of as many as six chromosomes. Some of these associations probably were due to reciprocal translocations. LOVE (1939) found numerous quadrivalent associations in *vulgare*-like derivatives of *vulgare-durum* crosses.

ORIGIN OF THE ABERRATIONS

Since no cytological study was made of the haploid which produced the aberrant plants, there is no direct evidence as to the mode of origin of the abnormalities. However, the observations of others, particularly of GAINES and AASE (1926) on a haploid of *T. compactum*, suggest several ways in which the aberrations could have arisen.

GAINES and AASE observed usually 21 univalents at first metaphase in their hybrid, although "occasionally two and rarely more" paired. The behavior of the chromosomes at first anaphase varied considerably. Sometimes the univalents were distributed to the two poles more or less at random; sometimes each univalent split and the halves went to opposite poles; and sometimes some of the univalents went as halves to opposite poles, while the halves of the rest failed to disjoin and went together to one or the other pole. In still other cells the entire chromosome group cohered in an irregular mass in the center of the cell. Micronuclei occurred frequently, as a result of chromosomes failing to be included in the telophase nuclei. The second division was observed to proceed more or less regularly if the chromosomes went to the poles as wholes at first anaphase, although supernumerary micronuclei frequently occurred. The behavior at second division was not determined for cells where all the univalents split at the first division nor where restitution nuclei were formed.

Origin of reciprocal translocations

KIHARA and NISHIYAMA (1937), KOSTOFF (1937), and HOWARD (1938) have pointed out that pairing between partially homologous chromosomes in hybrids may lead to the occurrence of segmental-interchange rings in the progeny of the hybrids. This explanation could apply also to haploids of *T. vulgare*, where a small amount of pairing occurs. GAINES and AASE (1926) reported occasionally one and rarely more pairs in their haploid, and RAW (1937) observed 0–5 bivalents and an occasional trivalent in *T. vulgare* haploids. Crossing over between a pair of non-homologous or partially homologous chromosomes (figure 10) would constitute a reciprocal translocation, and would result in a ring of four in backcrossed progeny if the two interchanged chromosomes were included in the same

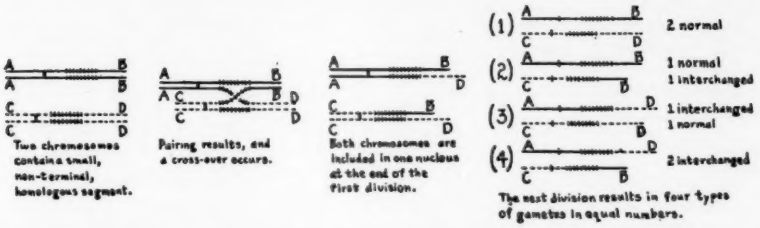
Two chromosomes contain a small, non-terminal, homologous segment.
Pairing results, and a cross-over occurs.
Both chromosomes are included in one nucleus at the end of the first division.
(1) 2 normal
(2) 1 normal 1 interchanged
(3) 1 interchanged 1 normal
(4) 2 interchanged
The next division results in four types of gametes in equal numbers.

FIGURE 10.—Probable method of origin of reciprocal translocations from a haploid of *Triticum vulgare*. A gamete of type 4, if fertilized by a normal germ cell, will produce a plant with a ring of four at meiosis, while types 2 and 3 will give a plant with a chain of four.

gamete. The formation of a single restitution nucleus such as was sometimes observed by GAINES and AASE at the end of the first division would permit both interchanged chromosomes to go to the same gamete. Or, if all the univalent chromosomes split and disjoined at the first division, the two chromosomes which were paired might disjoin tardily and both be included in the same telophase nucleus.

As emphasized by HOWARD (1938) and illustrated in figure 10, through random segregation of the four chromatids of a bivalent in which crossing over had occurred, only one-fourth of the gametes formed from spore-mother cells in which an interchange had taken place would have two interchanged chromosomes, one-fourth would be normal, and one-half would contain one normal and one interchanged chromosome. An egg of this last type would have a duplication and a deficiency, and if fertilized by a normal sperm, would produce a plant with a chain of four, as in figure 9. The fact that no such plant was discovered in the immediate progeny of the haploid may have been due to the difficulty of detecting the infrequent chains of four and trivalents which would have occurred if the deficient-duplicated segment was small.

Origin of monosomes and trisomes

These two abnormalities will be discussed together because every mode of origin to be suggested for one also applies to the other. Where only

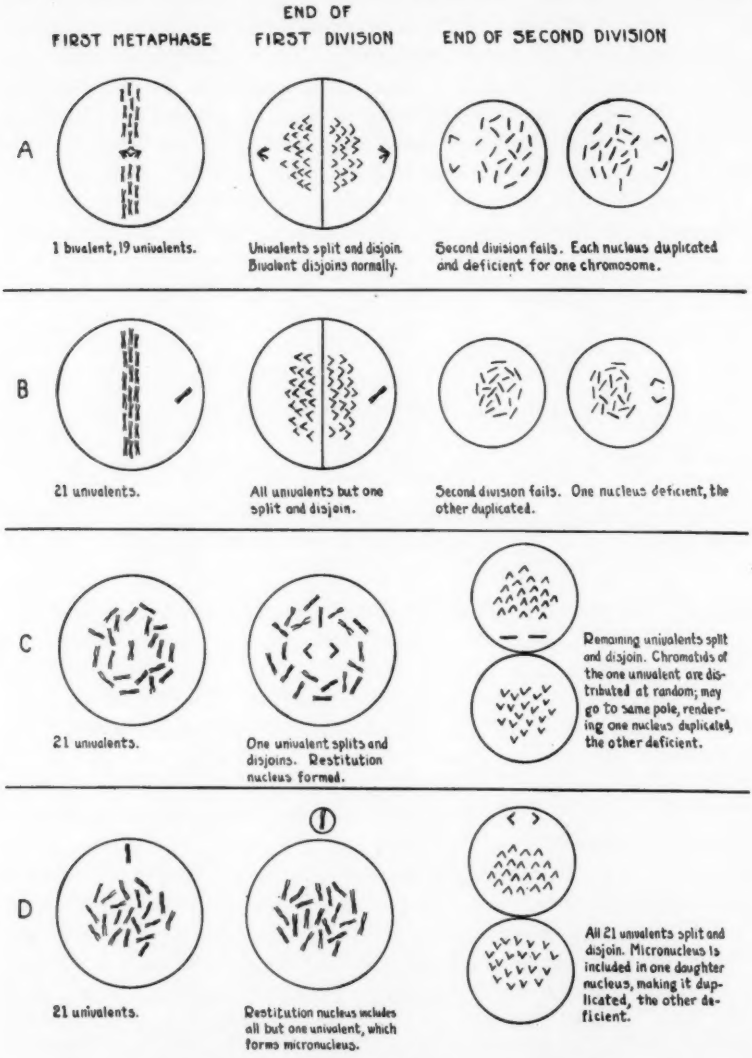
FIRST METAPHASE
END OF FIRST DIVISION
END OF SECOND DIVISION
A
1 bivalent, 19 univalents.
Univalents split and disjoin. Bivalent disjoins normally.
Second division fails. Each nucleus duplicated and deficient for one chromosome.
B
21 univalents.
All univalents but one split and disjoin.
Second division fails. One nucleus deficient, the other duplicated.
C
21 univalents.
One univalent splits and disjoins. Restitution nucleus formed.
Remaining univalents split and disjoin. Chromatids of the one univalent are distributed at random; may go to same pole, rendering one nucleus duplicated, the other deficient.
D
21 univalents.
Restitution nucleus includes all but one univalent, which forms micronucleus.
All 21 univalents split and disjoin. Micronucleus is included in one daughter nucleus, making it duplicated, the other deficient.

FIGURE 11.—Possible methods of origin of deficient and duplicated gametes from a haploid of *Triticum vulgare*.

monosomes occurred, as in plants 1 to 5 and in plant 7, it is possible that these arose through random segregation of 19 or 20 chromosomes to one pole at the first division in the haploid, a possibility which does not provide for the occurrence of trisomes. However, the chance of such an unequal distribution as 20:1 is so small that its occurrence even once among so few flowers as were pollinated would be highly improbable.

One way in which monosomes and trisomes could originate has already been suggested by VON BERG (1935), as a result of his observations on meiosis in a 21-chromosome generic hybrid, *T. turgidum*×*Haynaldia villosa*. He found, as had GAINES and AASE in their wheat haploid, that frequently all the univalents split and disjoined at the first division, and he observed that at the second division, restitution nuclei were formed which included all 21 chromatids. He pointed out (figure 11, A) that the presence and normal disjunction of a bivalent in a cell where all the univalents split and go to opposite poles at the first division would render the resulting gametes deficient for one member of the bivalent and duplicated for the other member. It should be pointed out that if an interchange occurred between the two paired chromosomes, the deficiency and duplication would be equal only to the non-interchanged parts of the chromatids, and a gamete with such a deficiency-duplication for a part of a chromosome, when fertilized by a normal germ cell, would produce a plant with a chromosome constitution like that in figure 9. Pairing in such a plant would depend on the size of the interchanged segments, and might never involve a chain of four if the segments were small.

Another possible explanation of the origin of monosomes and trisomes (figure 11, B) is based on the observation of GAINES and AASE that both halves of one or more univalents sometimes fail to disjoin at the first division and are distributed together to one or the other pole, while the other univalents split and go to opposite poles. A daughter nucleus which received both halves of a univalent would be disomic for that chromosome, and the other nucleus would be nullosomic.

Another type of division in the *T. compactum* haploid of GAINES and AASE which suggests the origin of monosomes and trisomes is the inclusion of all the chromosomes in a single, restitution nucleus following failure of the first division. If one of the univalents were already divided when the restitution nucleus was formed (figure 11, C), the two chromatids might be distributed independently at the next division (which presumably would be equational for the rest of the univalents) and frequently pass to the same daughter nucleus, making that nucleus duplicated and the other deficient.

A fourth method of origin (figure 11, D) was suggested by KIHARA and NISHIYAMA (1937) for disomic and nullosomic gametes produced by the

21-chromosome hybrid *T. polonicum*×*Haynaldia villosa*. Cytological observation showed that about 30 percent of pollen mother cells formed restitution nuclei at the end of the first division and that over a fourth of these restitution nuclei were accompanied by one or more micronuclei. Each micronucleus contained a single chromosome, and this chromosome presumably divided within its nucleus at the second division. The inclusion of a micronucleus in one of the telophase chromosome groups of the dividing restitution nucleus was suggested as an explanation for disomic gametes, while the omission of micronuclei explained the origin of deficient gametes.

Finally, there must be mentioned the possibility of an interchange occurring (as in figure 10) which was large enough that a gamete of type 2 or 3 (containing only one interchanged chromatid), upon fertilization by a normal gamete, would produce a plant in which a trivalent and a univalent were regularly formed, with never a chain of four. It is doubtful, however, that such a large interchange occurs other than very rarely. Most of the chromosomes of *T. vulgare* have median or near-median centromeres, so that interchanges on both sides of the centromere, each involving the same chromatid, would be necessary to make a chromatid much more than half interchanged. From the observable looseness of the association at metaphase in wheat haploids, it is unlikely that interchanges often occur on both sides of the centromere.

DISCUSSION

Several possibilities present themselves for the use of chromosome aberrants in a broad genetic analysis of *T. vulgare*. One possible study is of the immediate effects on the plant of deficiencies and duplications of chromosomes and parts of chromosomes. Another line of attack is to use monosomes and trisomes to locate on specific chromosomes the genes of common wheat.

Hybrids of the various aberrants with wheats of the emmer group will show at meiosis whether any of the chromosomes concerned are homologous with emmer chromosomes. As previously noted, five monosomes have thus far proved to have a homolog in the emmer complement. The hybrids which involve these monosomes can presumably be used to introduce, through backcrossing, single, intact emmer chromosomes into *T. vulgare*. Observation of the effects of these emmer chromosomes may shed light on the phylogeny of *T. vulgare*, and will have obvious practical applications. By backcrossing these same hybrids to the emmer parent, it should be possible to obtain monosomics in emmer, if they are viable, and to compare the effects of the deficiencies there with their effect in *T. vulgare*, where an additional set of seven chromosomes is present.

Homologies among the 21 chromosomes of *T. vulgare* are indicated by the formation of bivalents in the haploid. Little is known about these homologies. They may consist of duplicated segments scattered among all 21 chromosomes, or they may be duplications confined to a few chromosomes. Up to five meiotic pairs have been observed in a haploid, but there is no knowledge whether these five represent the total amount of homology, or whether they are merely the random result of homologies which involve more than ten chromosomes but which are too slight ever to result in more than five pairs in a single cell. Reciprocal translocations obtained from haploids of *T. vulgare*, if due to crossing over between homologous regions, furnish a means of determining the homology of definite chromosomes and regions. If association in the haploid is restricted to certain, partially homologous chromosomes, then the translocations which result should always involve the same chromosome combinations. These could be identified by crosses among a number of plants containing translocations of different origin, and by crosses with specific nullosomics or monosomics.

It is possible that many other supposedly polyploid plants will prove amenable to the same sort of analysis as that under way on *T. vulgare*. The results of STADLER (1931), KATAYAMA (1934), YEFEIKIN and VASILYEV (1936), GERASSIMOWA (1936), and IVANOV (1938), indicate that X-rays may be effective in inducing haploidy, and SINGLETON (1938) noted the occurrence of several haploids after ultra-violet treatment of maize pollen. Twin seedlings constitute another source of haploids (NAMIKAWA and KAWAKAMI 1934; HARLAND 1936; KIHARA 1936; YAMAMOTO 1936; MÜNTZING 1937, 1938; WEBBER 1938; KASPARYAN 1938). Whether haploids of other polyploid species than *T. vulgare* will give rise to the same types of chromosome aberrations remains to be seen.

Hybrids with little chromosome pairing might be studied with profit in the foregoing fashion. If an amphidiploid could be produced and backcrossed successfully to the hybrid, the resulting plants might then contain segmental-interchange associations and whole-chromosome deficiencies and duplications.

ACKNOWLEDGMENTS

The author is indebted to DR. L. J. STADLER for encouragement during the investigation, and to him and DR. BARBARA MCCLINTOCK for suggestions in the preparation of the manuscript.

SUMMARY

1. From a haploid of *Triticum vulgare*, 13 viable seeds were obtained by application of pollen from diploids.

2. Eleven of the resulting plants showed abnormal meiotic associations.

A total of 16 univalents, five trivalents, and two rings of four were present in the 11 individuals, the most aberrant of which had two univalents, two trivalents, and a ring of four.

3. Nearly all of the aberrant plants differed from normal in morphology, color, size, or vigor. Selfed seeds were obtained on all but one, and that one was fertile to pollen from a normal plant.

4. Nullosomic plants were obtained in the progeny of two monosomics. One of these was dwarfed and sterile, while the other was only semi-dwarf and was fertile.

5. Crosses of *T. durum* with eight monosomic plants of different origin showed that five of the eight involved a chromosome homologous to one of those of *T. durum*.

6. A tetrasomic plant, phenotypically indistinguishable from normal, was found in the progeny of a trisomic.

7. Rings of four were presumably the result of segmental interchanges which occurred in the haploid as crossovers between paired, partially homologous chromosomes.

8. From a plant with a ring of four, an individual with an unstable chain of four was obtained, supposedly as a result of the functioning of a deficiency-duplication gamete.

9. The various chromosome aberrations apparently resulted from some regulatory process in the haploid which favored the production of spores with near the somatic number of chromosomes. The use of these aberrations provides a new method for the genetic anlysis of *T. vulgare*—a method which may be applicable to polyploid plants in other genera.

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A PRELIMINARY SURVEY OF THE OENOTHERA ORGANENSIS POPULATION

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HABITAT AND DISTRIBUTION

OENOTHERA ORGANENSIS is known solely from the one range of mountains from which it takes its name. The Organ Mountains of southern New Mexico form part of a long chain lying east of and parallel to the Rio Grande. They differ from the San Andres Mountains on the north and the Franklin Mountains on the south by their greater altitude and in their volcanic origin. There has recently been published a Geology of the Organ Mountains (DUNHAM 1935) from which the accompanying map (figure 1) has been taken. The Organ Mountains occupy an area approximately eighteen miles long (north and south) and varying from three to eight miles in width, lying about fifteen miles east of Las Cruces, New Mexico. The mountains rise from a plain of four to five thousand feet elevation to a height of 9108 feet at Organ Peak. Of the area occupied by the mountains, approximately thirty-three square miles are above the 6000 feet level and less than ten square miles above the 7000 feet level. Oenotheras have been found only at elevations above 6000 feet and only in canyons which drain relatively large areas of higher land.

The canyons in which the oenotheras are found have been carved out of the solid igneous rock, rhyolite, which makes up the greater part of the mountains. Both the side walls and the floors of the canyons are for the most part very steep, so that the runoff of water after rains is extremely rapid. The rainfall in the mountains has not been measured but is perhaps not much greater than at State College, near Las Cruces, where the mean annual precipitation in a period of over seventy years is approximately eight and one-half inches. The largest amount of precipitation occurs in the summer months and generally as local thunder showers. A large part of the mountains is definitely arid, and with the exception of Filmore Canyon, which has a permanent stream, all the water courses are dry most of the time. While the canyons were being formed, however, basins were eroded in the canyon floors. These have become filled with rock and gravel and it is in these natural reservoirs that the oenotheras are found. Growing with the oenotheras in these pockets is an Aquilegia, whereas the immediate environs are occupied by such xerophytes as Yucca, Agave, Opuntia, etc. These conditions are illustrated in the photographs in figure 2.

Oenothera organensis, unlike the other oenotheras with angled seeds (that is, the onagras), is strictly perennial. The central axis never elongates,

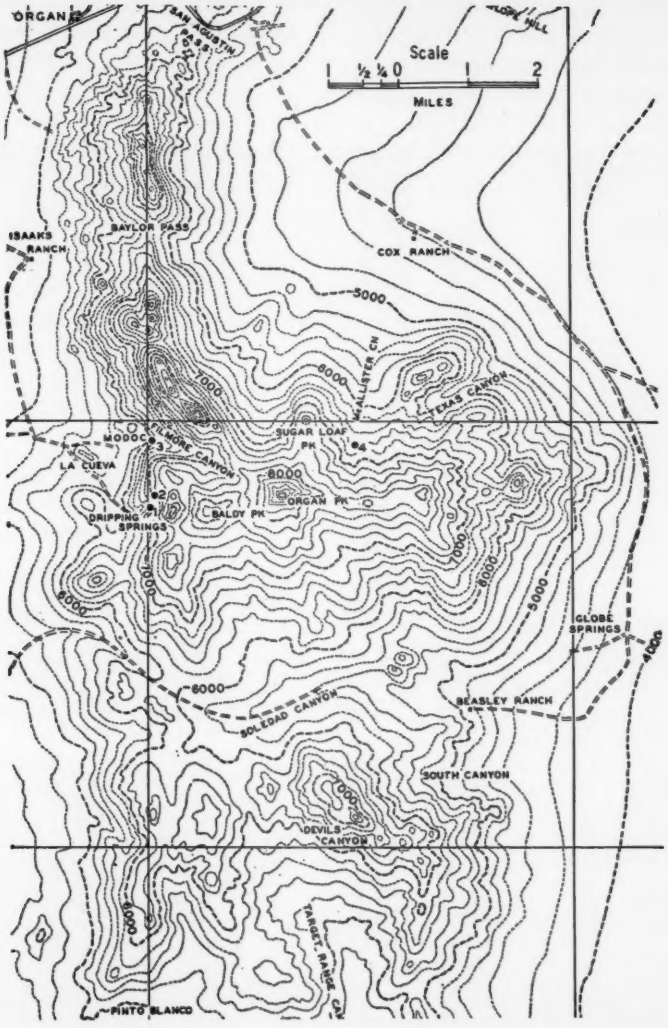

FIGURE 1.—Topographical map of the Organ Mountains—after DUNHAM (1935) in the Bulletin of the New Mexico School of Mines. The original is one inch to the mile and shows the geological formations.

but all stems are lateral branches arising from the axils of the rosette leaves, or from adventitious buds produced on the roots. Instead of a single tap root there are numerous "fibrous" roots varying from one-eighth

to three-quarters of an inch in diameter and usually attaining a length of about two feet in the first season. The plants are capable of withstanding severe drought, but under such conditions there is little growth and no flowering. In fact, under our conditions of culture, the water requirements

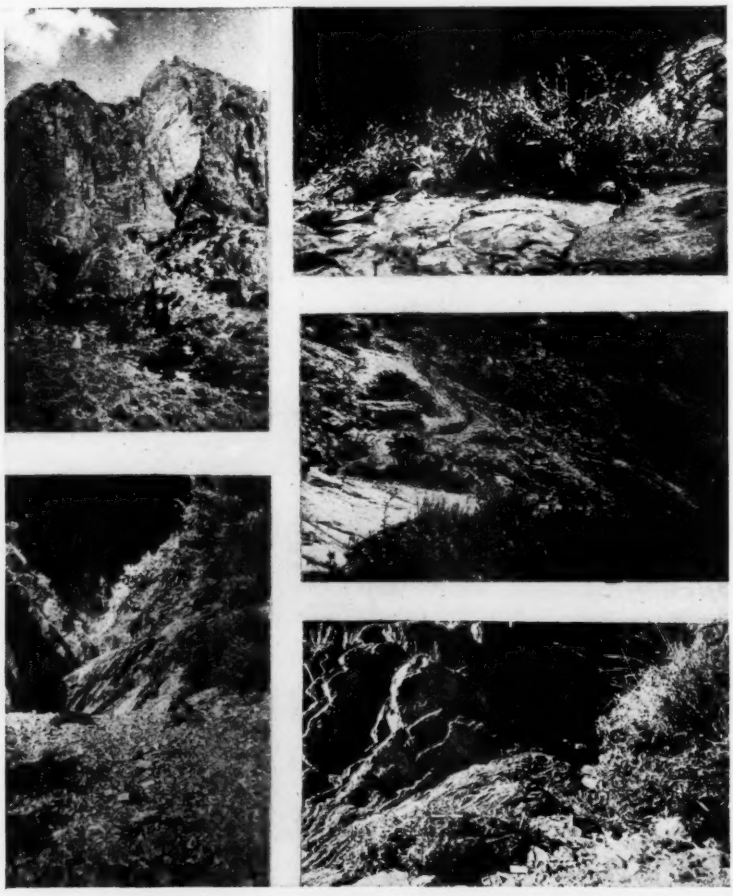

FIGURE 2.—Upper left: rhyolite cliffs at Dripping Springs, the East Fork runs slightly to the right of the center of the photograph, the North Fork is at right angles and only the base is included in this photograph; upper right: portion of a group of twenty-two plants of *Oenothera organensis* in the upper part of the North Fork; right center: the same group from the opposite side showing yuccas and cacti in the near background; lower left: water-course in the East Fork with one oenothera shown; lower right: higher in the same canyon, the oenothera in the foreground has been flattened by a recent runoff of water. Upper right photograph by D. G. CATCHESIDE.

for flowering in this species are very much greater than in other oenotheras. An instance of the failure of plants growing in their normal habitat to flower because of drought was observed in a small canyon on the north slope of the mountains (number 4 in figure 1). A population of about 20 plants was found there in September, 1937. All plants showed the effects of drought in their burned leaves and arrested growth, and only one had flowered that season whereas six still bore seed capsules from the previous year.

The foregoing is an account of the conditions to which *Oenothera organensis* is adapted. I have not searched other mountain ranges in the region for this species, but it has never been reported outside of this one range, and the lesser altitudes and the sedimentary origins of the near-by mountains make it seem improbable that they support populations of this species.

Within the Organ Mountains, *Oenothera organensis* has been found in four localities. The largest stand (114 plants) so far discovered is at Dripping Springs, the type locality. The first collections were made in 1881 by VASEY, 1894 to 1899 by WOOTON, 1898 by COCKERELL, 1906 by STANDLEY, as reported by WOOTON and STANDLEY (1913) under the name *Oe. macrosiphon*, and a later collection was made by PROFESSOR P. A. MUNZ in 1935. DOCTOR D. G. CATCHESIDE and I visited this locality in June, 1937, collecting seeds and cuttings, and I returned in September of that year for further collections. The oenotheras occur in two forks of the canyon (numbers 1 and 2 in figure 1). In the East Fork there were 43 plants growing in ten locations having respectively 1, 4, 4, 1, 2, 9, 1, 9, 5 and 7 plants and spread over about 800 feet of the water course. This fork is the source of the material collected by MUNZ (Emerson 1938). In the North Fork, 71 plants occur in seven locations having respectively 4, 2, 3, 22, 9, 19 and 12 plants each, and spread over about 400 feet of the water course. The populations in the two forks are over 600 feet apart and separated by a ridge 300 to 400 feet high. Two smaller forks of the canyon, extending to the southeast and southwest respectively, were devoid of oenotheras. In September the plants had been severely beaten by the runoff of a recent rain, but as near as could be judged, 47 of the plants at Dripping Springs had flowered and set seed, 38 others had apparently flowered but no capsules were found, and the remaining 29 (mostly young plants) had evidently not flowered that season. All but 19 of the plants then at Dripping Springs are now represented in the garden at Pasadena, either as plants grown from cuttings from the original plants or by the progenies from open-pollinated capsules.

Modoc Waterfall (number 3 in figure 1) is about a mile from Dripping Springs from which it is separated by high land, though it is possible that

the upper extreme of the North Fork of the canyon at Dripping Springs is narrowly separated from the upper part of Filmore Canyon. A previous collection from this locality had been made by DR. E. W. ERLANSON and DR. A. E. ARCHER in 1928 (EMERSON 1938). In June, 1937, there were about 20 plants growing on the cliff on the north side of the waterfall where they were watered by the mist from the fall. Cuttings were made from 14 of these plants (all that could be reached without ropes) and are now growing in Pasadena. DOCTOR CATCHESIDE and I explored Filmore Canyon for about half a mile above Modoc Waterfall and found only one straggling plant growing about five hundred yards above the falls. The canyon above the falls has a fairly level floor, running water and very much more vegetation than other canyons visited. It is possible that the oenotheras can not compete with other plants in the moister habitat, but it is also possible that oenotheras once grew here but have been destroyed by cattle which now graze this canyon. Filmore Canyon penetrates deeply into the mountains and may support oenothera colonies in the upper portions.

The oenotheras in McAllister Canyon (number 4 in figure 1) are about three miles distant from the localities reviewed above, from which they are separated by the highest part of the range. About 20 plants were found scattered along a very steep and rocky water course in one branch of the canyon. Seeds were collected in September, 1937, from the six plants on which capsules remained from the previous year.

McAllister Canyon was selected as the most promising region in which to find oenotheras on the northern slope after driving around the northern half of the mountains (Dripping Springs to Beasley Ranch in figure 1). The entire northern spur and the east slope from Texas Canyon to Beasley Ranch appeared much too dry to support oenotheras. The vegetation at the mouth of McAllister Canyon indicated a greater runoff of water than at other points, which suggested it as a favorable locality. It is probable that stands of *Oenothera organensis* are to be found in other branches of McAllister Canyon and presumably in the canyon on the west of Sugar Loaf Peak and in some of the canyons extending from Soledad Canyon back towards Organ Peak. On the basis of this preliminary survey, it is estimated that the entire population of this species consists of less than one thousand plants and very likely less than five hundred.

IDENTIFICATION OF SELF-STERILITY ALLELOMORPHS

The cuttings from Dripping Springs and from Modoc were rooted by the indole-acetic acid technique (COOPER 1936). The resulting plants were kept in the greenhouse over winter and treated with extra hours of illumination. Those which flowered were tested for sterility allelomorphs

and later the whole collection was transferred to the field, where they suffered a severe set-back and few additional plants flowered during the summer. Cultures were grown from seed collected at Dripping Springs and McAllister Canyon, and these flowered more freely.

The method used to identify self-sterility allelomorphs has been described in an earlier paper (EMERSON 1938). Following appropriate pollinations, the growth of pollen tubes in the stylar tissue is observed. Pollen carrying a particular allelomorph produces extremely short tubes in styles also carrying that allelomorph, but produces normally growing tubes in any style not carrying that allelomorph. Plants of known constitutions had been retained from previous years and were used as testers. New testers were established as rapidly as additional allelomorphs were found.

The raw data showing the results of these tests are too cumbersome to publish as they involve other 3000 crosses between approximately 100 different genotypes.[1] The data presented at this time show the distribution of genotypes in the progenies of open-pollinated capsules collected in the wild (table 1) and the distribution of the different self-sterility allelomorphs in the four localities from which collections were made (table 2). The additional observations reported at this time are in agreement with the genetic results previously reported (EMERSON 1938). All self-sterility genes so far determined, and there are now 37, are apparently different mutations at a single locus, that is they are strictly allelomorphic. In testing individual plants, pollen carrying many different allelomorphs was used, and in each instance pollen carrying two and only two allelomorphs failed to produce normally developing pollen tubes in the styles of the plant tested. In the cultures derived from open pollinated capsules it is apparent that two and only two allelomorphs were derived from the maternal parent. No allelomorph has been found which permits normal pollen tube growth following self-pollination.

The purpose of growing seeds from open pollinated capsules was in part to determine the allelomorphs carried by plants from which no cuttings were rooted and in part to determine the number of kinds of pollen taking part in the pollination of single flowers. To insure the identification of allelomorphs carried by the seed parent, seeds from more than one capsule from each plant were sown, since if a single capsule resulted from pollination by a single pollen parent there could be no more than four allelomorphs represented in the progeny and it would be impossible to tell which were of maternal and which of paternal origin. One culture (listed under #100 in table 1) illustrates this point. Plants in this culture carried four

[1] I am indebted to MR. GEORGE T. RUDKIN for the preparation of most of the styles used in these tests. By improving the dissection technique, he was able to make as many as 45 completed preparations in an hour, which was twice the previous rate and greatly accelerated the tests.

TABLE 1

Frequencies of genotypes in progenies from open-pollinated capsules. The italicized numerals refer to specific self-sterility allelomorphs, x and y to incompletely identified allelomorphs which, however, are distinct from all other allelomorphs recorded in the same progeny.

McAllister Canyon:

#1	SEEDS FROM 1 CAPSULE				SEEDS MIXED FROM 5 CAPSULES				
♀\♂	*26*	*31*	*x*	*y*	*6*	*16*	*29*	*x*	*y*
2	—	1	1	1	1	1	—	—	—
25	3	—	—	—	—	—	1	1	1

#2	1 CAPSULE						1 CAP.		1 CAPSULE		
♀\♂	*2*	*14*	*19*	*29*	*33*	*x*	*2*	*35*	*14*	*34*	*x*
9	—	4	1	—	1	1	1	2	1	3	—
31	2	—	1	1	3	—	1	—	—	2	1

#3	1 CAP.		5 CAPSULES			
♀\♂	*x*	*y*	*7*	*13*	*28*	*30*
27	1	—	—	1	1	—
31	—	1	1	—	—	1

#4	1 CAPSULE						3 CAPS.	
♀\♂	*11*	*12*	*25*	*37*	*x*	*y*	*9*	*34*
19	1	1	1	1	1	—	—	1
31	1	1	1	3	1	1	1	—

#5	5 CAPSULES		
♀\♂	*13*	*x*	*y*
37	2	1	1
38	2	—	—

#6	7 CAPSULES			
♀\♂	*12*	*34*	*x*	*y*
13	1	1	—	—
37	—	—	1	1

East Fork, Dripping Springs:

#100	1 CAP.		1 CAP.
♀\♂	*12*	*20*	*5*
11	2	5	1
19	2	3	—

TABLE 1—(*Continued*)

#104	1 CAP.		1 CAPSULE				
♀\♂	7	x	4	12	18	21	31
26	1	—	1	1	1	1	—
35	—	1	1	—	—	—	2

#105	1 CAPSULE					1 CAPSULE			
♀\♂	3	12	21	37	x	3	39	x	y
7	—	—	1	1	—	1	1	1	1
24	1	1	—	—	1	—	1	—	—

#106	1 CAP.		1 CAP.	
♀\♂	5	25	13	22
7	2	1	—	2
24	1	4	3	—

North Fork, Dripping Springs:

#215	1 CAP.	1 CAPSULE		
♀\♂	36	7	13	x
9	—	1	—	1
29	1	—	1	—

#261	1 CAPSULE				1 CAP.
♀\♂	7	17	x	y	x
22	1	1	—	—	1
29	—	—	1	1	—

#267	1 CAPSULE							1 CAPSULE			
♀\♂	15	19	22	30	36	x	y	3	7	33	x
20	—	—	—	—	—	—	—	—	—	1	1
29	1	1	2	1	2	1	1	1	1	3	—

#269	1 CAP.		1 CAP.
♀\♂	29	x	35
3	2	1	1
20	1		—

TABLE 2

Gene frequencies of 34 self-sterility allelomorphs in four localities in the Organ Mountains.

ALLELOMORPHS	MCALLISTER CN			DRIPPING SPRINGS						MODOC	TOTALS		
				EAST FORK			NORTH FORK						
	♀♀	♂♂	TOTAL	♀♀	♂♂	TOTAL	♀♀	♂♂	TOTAL	♀♀	♀♀	♂♂	TOTAL
2	1	2	(3)	—	—		—	—		—	1	2	(3)
3	—	—		1	2	(3)	3	—	(3)	—	4	2	(6)
4	—	—		—	1	(1)	—	—		4	4	1	(5)
5	—	—		1	2	(3)	—	—		—	1	2	(3)
6	—	1	(1)	—	—		—	—		—	—	1	(1)
7	—	1	(1)	2	1	(3)	—	3	(3)	—	2	5	(7)
9	1	1	(2)	—	—		2	—	(2)	—	3	1	(4)
11	—	1	(1)	2	—	(2)	2	—	(2)	—	4	1	(5)
12	—	2	(2)	1	3	(4)	1	—	(1)	—	2	5	(7)
13	1	2	(3)	—	1	(1)	1	1	(2)	—	2	4	(6)
14	—	2	(2)	2	—	(2)	—	—		3	5	2	(7)
15	—	—		—	—		3	1	(4)	—	3	1	(4)
16	—	1	(1)	3	—	(3)	—	—		—	3	1	(4)
17	—	—		1	—	(1)	—	1	(1)	—	1	1	(2)
18	—	—		5	1	(6)	—	—		—	5	1	(6)
19	1	1	(2)	1	—	(1)	1	1	(2)	—	3	2	(5)
20	—	—		—	1	(1)	5	—	(5)	—	5	1	(6)
21	—	—		—	2	(2)	3	—	(3)	—	3	2	(5)
22	—	—		—	1	(1)	3	1	(4)	—	3	2	(5)
24	—	—		2	—	(2)	—	—		—	2	—	(2)
25	1	1	(2)	—	1	(1)	—	—		—	1	2	(3)
26	—	1	(1)	1	—	(1)	—	—		—	1	1	(2)
27	1	—	(1)	—	—		—	—		—	1	—	(1)
28	—	1	(1)	—	—		—	—		—	—	1	(1)
29	—	2	(2)	1	—	(1)	3	1	(4)	—	4	3	(7)
30	—	1	(1)	—	—		—	1	(1)	—	—	2	(2)
31	3	1	(4)	—	1	(1)	—	—		—	3	2	(5)
33	—	1	(1)	—	—		—	1	(1)	—	—	2	(2)
34	—	3	(3)	—	—		—	—		—	—	3	(3)
35	—	1	(1)	1	—	(1)	1	1	(2)	—	2	2	(4)
36	—	—		—	—		3	2	(5)	—	3	2	(5)
37	2	1	(3)	—	1	(1)	—	—		—	2	2	(4)
38	1	—	(1)	—	—		—	—		—	1	—	(1)
39	—	—		—	1	(1)	—	—		—	—	1	(1)

combinations of four allelomorphs: *11/12*, *11/20*, *12/19* and *19/20*.[2] This distribution of allelomorphs is characteristic of the progeny of a cross between two plants, one carrying allelomorphs *11/19*, the other *12/20*, but which was the maternal and which the paternal parent can not be determined from this distribution alone. Another capsule from the same seed-parent produced a plant of the constitution *5/11*. In this plant allelomorph *11* is the only one that could have been derived from the maternal parent which must consequently be *11/19*. Table 1 is arranged to show which allelomorphs were derived from the maternal and which from the paternal parents in each instance.

In table 2, under the heading ♀ ♀, are listed the number of plants carrying particular allelomorphs as determined directly from the cuttings or by progeny tests. The number of capsules in which a particular allelomorph was contributed by the pollen parent are listed under the heading ♂ ♂. It is realized that the number of pollinations in which a particular allelomorph takes part should not correspond exactly to the frequency in the population of plants carrying that allelomorph, but it may give a fairly good approximation. It should also be noted that the pollen sampled from McAllister Canyon was produced in 1936, that from Dripping Springs in 1937.

DISTRIBUTION OF ALLELOMORPHS BETWEEN CANYONS

The distributions shown in table 2 seem to indicate that the samples collected in different canyons represent parts of a single interbreeding population. The data from the three localities best sampled are summarized in the following table. Allelomorphs obtained from the 1935 collection have already been reported (EMERSON 1938).

	1937 COLLECTION	INCLUDING COLLECTION OF 1935
McAllister only	6	5
East Fork only	5	7
North Fork only	2	2
McAllister and East Fork only	6	7
McAllister and North Fork only	3	2
East Fork and North Fork only	5	5
All three localities	7	8
Totals	34	36

[2] Self-sterility allelomorphs are customarily designated by the symbols S_1, S_2, S_{11}, etc., but it has been found that the small subscripts do not adequately distinguish the different allelomorphs. For this reason the numeral subscripts alone are being used in this paper and are italicized to distinguish them from other numerals used to designate frequencies, etc. The symbol S_{11} becomes simply *11*, the fractional form *11/12* represents a plant carrying S_{11} and S_{12}.

Thirteen allelomorphs (or 14 if the 1935 collection is included) have each been recovered from but one locality, 13 (or 15) are common to McAllister Canyon and the East Fork of Dripping Springs, 10 are common to McAllister Canyon and the North Fork of Dripping Springs, and 12 (or 13) are common to the east and North Forks of Dripping Springs. The similarities between the three canyons are apparently equally strong and are as great as would be expected in samples of such sizes from a uniform population.

The sample reported from Modoc Waterfall is still very small. Allelomorph *4* is common to Modoc and the East Fork of Dripping Springs and allelomorph *14* is common to Modoc, McAllister and the East Fork of Dripping Springs. One plant from Modoc is known to carry either *15* or *22*, both of which are known from the North Fork of Dripping Springs, so that Modoc has allelomorphs in common with each of the other localities sampled. The collection made at Modoc in 1928 (EMERSON 1938) contained allelomorphs *1*, *2*, *3* and *4*. The first has not turned up again, but allelomorph *2* was found in McAllister Canyon, *3* in both forks of Dripping Springs, and *4* is present in the East Fork of Dripping Springs and is apparently the commonest allelomorph still at Modoc.

DISTRIBUTION OF ALLELOMORPHS IN SMALL LOCAL REGIONS

The group of plants growing highest in the East Fork of Dripping Springs contains seven plants, of which five have been completely identified. These have the following constitutions: *14/18*, *16/17*, *16/18*, *14/18* and *14/18*. These constitutions suggest that the entire group was derived from a single seed parent, perhaps *14/16* or *17/18*. A group of plants lower in the same canyon contains allelomorphs *3*, *5*, *11*, *12*, *18* and *29*, no one of which has so far been found in more than a single plant in this locality, indicating a more diverse origin than that of the group higher in the canyon.

The group highest in the North Fork is composed of 12 plants of which only three have been completely determined. These have the constitutions *22/29*, *20/29* and *3/20*, again suggesting a close ancestral relationship. A group of 22 plants lower in the same canyon has given the following constitutions: *11/36*, *11/36*, *9/29*, *15/22*, *3/22*, *15/x*, *9/35* and *12/13*, indicating a certain degree of ancestral relationship, but also considerable diversity.

Five plants of the population of about 20 growing at Modoc Waterfall have been incompletely identified. These have the constitutions *4/14*, *4/x*, *4/y*, *14/z* and *4/14*, suggesting a very close ancestral relationship. It should be remembered that allelomorph *4* was known to be present in this immediate locality nine years earlier.

The one branch of McAllister Canyon visited had about 20 plants which were fairly well scattered, but which were also well isolated from other stands. The six plants identified from this locality have the following constitutions: *2/25*, *9/31*, *27/31*, *19/31*, *37/38* and *13/37*. All but one of these plants had either allelomorph *31* or *37*.

The data so far obtained seem to indicate that seed dispersal is brought about principally by water and accordingly takes place in a down-stream direction for the most part. Plants growing at the tops of canyons or in otherwise isolated groups would often be descended from plants previously occupying the same location and should show close relationships.

The transfer of allelomorphs from one canyon to another is likely accomplished through the pollen, though some transfer of seeds is to be expected. There were apparently but six plants in one branch of McAllister Canyon that flowered in 1936 and these plants carried only nine allelomorphs. The pollen functioning on them carried 13 additional allelomorphs that presumably originated in other localities. Seeds are probably carried by birds, but the likelihood of their falling in favorable spots is less than when carried by water.

TYPES OF POLLINATIONS

Oenothera organensis, like other members of the genus, is presumably insect pollinated, but the particular insect effective in this instance is not known. The flowers are open and receptive to pollination from early dusk till a few hours after sunrise. The conditions suggest that some strong-flying, nocturnal insect, such as a sphinx moth, is a likely agent. Against this supposition is the observation that sphinx moths regularly visit other species of oenothera in the garden at Pasadena but do not bring about the pollination of *Oe. organensis*. It is estimated that less than 50 open-pollinated capsules have set on the 500 or more plants of this species that have flowered in the garden during the last two seasons. In the Organ Mountains, on the other hand, there is an abundant set of capsules, even on isolated plants. Since the hypanthium in this species is so long, averaging six inches or longer, a sphinx moth with a tongue of suitable length was sought in the Organ Mountains, but none of this sort was attracted to our light on the two evenings on which attempts were made. The difficulties of descending after dark from the relatively inaccesible locations of freely flowering groups of oenotheras discouraged us from making direct observations on the pollinating agents, but such observations will probably be necessary to settle the point.

The data presented in table 1 tell something of the manner in which pollinations take place even though the means by which they occur is unknown. It was to be expected that most of the pollen deposited on a

stigma would come from the flower previously visited by the pollinating agent. The pollen should often come from another flower on the same plant, resulting in self-pollination from which no seeds would set. In case the pollen came from a plant of different genetic constitution, only two allelomorphs should be carried by the pollen. Cultures from four open-pollinated capsules (one capsule listed under each of #2 and #100 and both capsules under #106 in table 1) indicate that this type of pollination does occur. It should be noted that each of the plants from which these capsules were collected was growing at some distance from other plants then in flower. Most of the other tests indicate that more than two allelomorphs are usually present in the pollen deposited on any one stigma. Thirteen plants tested from one capsule (see #4, table 1) showed six allelomorphs present in the functioning pollen, and nine plants tested from another capsule (see #267) showed seven allelomorphs in the functioning pollen. It is expected that further tests will show that a large number of allelomorphs are usually present in the pollen functioning on any one stigma. Two means are suggested by which this result could be accomplished: either the pollinating insect brings a mixture of pollen from many flowers, or the flowers must be visited repeatedly, with pollen from more than one visit functioning.

FREQUENCIES OF ALLELOMORPHS

The sample of allelomorphs so far identified is too small to show the relative frequencies of different allelomorphs in the native population. There is still little agreement between the frequencies of allelomorphs recovered through the pollen and of those carried by the plants so far sampled. The data now available are shown in table 2.

ACKNOWLEDGMENTS

I am indebted to Dr. E. H. Wells, Director of the New Mexico School of Mines, Socorro, N. Mex., for permission to reproduce the topographical map appearing as figure 1 in this paper. Doctor and Mrs. T. C. Sexton of Las Cruces kindly permitted me to camp at Dripping Springs and take cuttings from the oenotheras in that region. Doctor A. L. Hershey, botanist at the State Agricultural College was very helpful in directing us to likely sites in which to find the oenothera, and Professor Crawford of the same institution kindly inspected the cuttings collected which aided us in passing the various state horticultural inspections.

SUMMARY

Oenothera organensis has been recorded only from the Organ Mountains of southern New Mexico. The particular type of habitat in which it is found and the water requirements for normal flowering are such that it

seems unlikely that the species occurs in other mountains in that region. The entire population is believed to consist of very few plants, perhaps not more than 500.

The samples so far analyzed show that the oenotheras growing in different canyons form part of a single, freely interbreeding population. The indications are that seed dispersal is brought about principally by water and is chiefly in a down-hill direction. The interchange of self-sterility allelomorphs between canyons is believed to be accomplished chiefly through the pollen. The means by which the pollen is carried has not been determined, but individual flowers have been shown to be pollinated by a mixture of pollen coming from many plants. The sample so far analyzed is insufficient to indicate the relative frequencies of different self-sterility allelomorphs.

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THE DISTRIBUTION OF SELF-STERILITY ALLELES IN POPULATIONS

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THE phenomenon of self-sterility is known to be very common among higher plants. The genetic mechanism in most cases studied seems to be that discovered by EAST and MANGELSDORF in *Nicotiana alata* and *N. Sanderae*. A single series of alleles, $S_1, S_2 \cdots$, is involved. The growth of a pollen tube is so much inhibited in a style whose cells contain the same allele that it does not reach the embryo sac. It is obvious that all individuals must be heterozygous and that a population cannot persist with less than 3 *S*-alleles. It is also fairly obvious that selection would tend to increase the frequency of any additional alleles that may appear. DR. STERLING EMERSON has been investigating a series of at least 37 self-sterility alleles of this sort, found in *Oenothera organensis*, a species in which the entire population apparently consists of less than one thousand individuals, scattered in small groups among certain canyons of the Organ Mountains of New Mexico (EMERSON 1938, 1939). I am indebted to him for calling my attention to the interesting questions involved in the effects of selection and inbreeding in this case.

SELECTION PRESSURE

We assume the existence of a series of n self-sterility alleles, $S_1, S_2 \cdots S_n$, with frequencies $q_1, q_2 \cdots q_n$ such that $\Sigma q = 1$ in a population of N diploid individuals. The frequencies of zygotes containing one of these (S_i) must be $2q$ (with the appropriate subscript) since all are heterozygotes. The frequency of functioning S_i female gametes is q, assuming no differential selection. The frequency of functioning S_i pollen grains is not in general the same. S_i pollen has, by hypothesis, no chance of functioning in the styles of zygotes containing S_i, but has a better than average chance in zygotes that lack S_i (frequency $1-2q$) since each zygote of this class inhibits pollen of two of the other kinds. Assume that on non-S_i styles, the ratio of successful S_i pollen grains to successful ones of other types is as $q:R(1-q)$. The total frequency of functioning S_i pollen is then $q(1-2q)/[q+R(1-q)]$. The average frequency of functioning S_i gametes is $q(1-q)(1+R)/2[q+R(1-q)]$ and the change from the previous generations is therefore

$$\Delta q = \frac{q(1-q)(1+R)}{2[q+R(1-q)]} - q = \frac{q}{2}\left[\frac{(1-R)-q(3-R)}{R+q(1-R)}\right].$$

Letting $\hat{q}$ represent the point at which selection has no effect ($\Delta q = 0$),

$$\hat{q} = \frac{1 - R}{3 - R}, \qquad R = \frac{1 - 3\hat{q}}{1 - \hat{q}}, \qquad \Delta q = -\frac{q(q - \hat{q})}{1 - 3\hat{q} + 2\hat{q}q}.$$

If all non-S_i alleles have the same frequency, it is obvious that R will have the value $(n-3)/(n-1)$ since each non-S_i style permits functioning of only $n-3$ of the $n-1$ types of non-S_i pollen grains. This leads to a formula for the frequency of functioning S_i gametes, $q(1-q)(n-2)/(n-3+2q)$ communicated to me by DR. EMERSON for this case. But the frequencies of the various alleles may be expected to drift at random about the equilibrium point as a result of the accidents of sampling. The average value of R will consequently be somewhat less than $(n-3)/(n-1)$ (unless $n=3$, $R=0$) since pollen grains with the more abundant alleles will be inhibited in excess. We will assume that R may be treated with sufficient accuracy as constant in populations that have long been subject to the same conditions (number of individuals, mutation rate in the S series, rate of cross breeding, etc.).

The fluctuating variations in R due to chance variations in the relative frequencies among the non-S_i alleles are not an important qualification but variations correlated with differences in the frequency of S_i itself introduce a systematic error. If there are only four alleles altogether, it is obvious that the three non-S_i alleles may be expected to be somewhat more nearly equal in frequency as a result of selection, if S_i is rare, than if it is common; and that R will consequently be negatively correlated with, q. The upward pressure on rare alleles being approximately $q(1-R)/2R$ the assumption that R is constant slightly overestimates the upward selection pressure on rare alleles carried by a population under given conditions. This effect rapidly decreases in importance as n becomes greater than 4.

MUTATION AND MIGRATION PRESSURES

Mutation from S_i at the rate u per generation, and to it at the rate v, changes gene frequency at the rate $v(1-q)-uq$. Crossbreeding between the population in question and the species as a whole, such that the proportion m of the gametes come from the latter, changes gene frequency at the rate $-m(q-q_i)$ where q_i is the frequency of S_i in the species as a whole. The net rate of change of gene frequency and its sampling variance are as follows:

$$\Delta q = \frac{q(1 - q)(1 + R)}{2[R + q(1 - R)]} - [1 + u + m(1 - q_i)]\, q + (v + mq_i)(1 - q)$$

$$\sigma^2_{\Delta q} = \frac{q(1 - q)}{2N}.$$

THE DISTRIBUTION FORMULA

The general formula for the distribution of gene frequencies determined by any systematic evolutionary pressure Δq, and random variations measured by $\sigma^2_{\Delta q}$, has been shown to be as follows (WRIGHT 1938).

$$\phi(q) = \frac{Ce^{2\int (\Delta q/\sigma^2_{\Delta q})dq}}{\sigma^2_{\Delta q}}.$$

Substituting the values of Δq and $\sigma^2_{\Delta q}$ found for the present case we have

$$\phi(q) = C[R + q(1 - R)]^{2N(1+R)/(1-R)} q^{4N(v+mq_1)-1}(1 - q)^{4N[1+u+m(1-q_1)]-1}.$$

The simplest special case is that of three alleles with negligible rates of mutation and cross breeding. In this case $R = 0$ since S_2S_3 can be fertilized only by S_1 pollen. Thus $\hat{q} = \frac{1}{3}$, $\Delta q = (1 - 3q)/2$.

$$\phi(q) = \frac{\Gamma(6N)}{\Gamma(2N)\Gamma(4N)} q^{2N-1}(1 - q)^{4N-1}.$$

The mean and variance of this distribution are easily found.

$$\bar{q} = \int_0^1 q\phi(q)dq = \tfrac{1}{3}$$

$$\sigma^2_q = \int_0^1 (q - \bar{q})^2\phi(q)dq = \frac{2}{9(6N + 1)}.$$

The mode is at the point at which

$$\frac{d \log \phi(q)}{dq} = 0$$

$$\text{Mode} = \frac{2N - 1}{6N - 2}.$$

These formulae can easily be generalized to allow for mutation and cross-breeding but not for the presence of a larger number of alleles. However, if values of N, u, v, m, q_1 and R are assumed, the ordinates of the distribution may be calculated as multiples of C. The value C required to make the total frequency one can then be found after estimating the sum of all frequencies. The mean ($\bar{q}$) and other statistics of the distribution can also be calculated empirically. Having found $\bar{q}$, the number of alleles (n) present under the conditions is given by the reciprocal ($\bar{q} = \Sigma q/n = 1/n$). The proportion of the alleles lost in each generation by the accidents of sampling can be estimated from the frequencies of the low gene frequencies $1/2N, 2/2N \cdots$, by use of the Poisson series, a question discussed in the next section. At equilibrium, losses must be balanced by accessions of new alleles by mutation or outbreeding. If the values of u, v or m, so calculated,

do not agree with those originally assumed and these are large enough to affect appreciably the form of the distribution, it becomes necessary to try other values of R until, by repeated trial and interpolation, agreement is reached.

THE RELATIONS BETWEEN MUTATION RATE, POPULATION SIZE AND NUMBER OF ALLELES

It has been shown previously (WRIGHT 1931) that the rate of loss of alleles is usually about half the frequency of the subterminal class, thus, $K=\frac{1}{2}f(1/2N)=(1/4N)\phi(1/2N)$. In the present case, however, there may be such strong selection pressure tending to increase the frequency of rare alleles that this formula requires modification. It is indeed obvious that it is impossible to lose an allele if only three are present without extinction of the population. If in this case q_1 happens to fall very low at any time (population nearly 100 percent S_2S_3), q_1 automatically rises almost to the opposite extreme ($q_1=.50$) in the next generation. For larger values of n, Δq may be taken as $\hat{q}\,q/(1-3\hat{q})$ where q is small. As $\hat{q}$ is close to $\bar{q}(=1/n)$ except in extreme cases, we may take $\Delta q=q/(n-3)$ as a rough approximation for small q and $n>3$. The chance of loss with a given small value of q is $e^{-2N(q+\Delta q)}$ or $e^{-2Nq(n-2)/(n-3)}$ approximately, making allowance for selection. On testing different extreme types of distribution (such as $\phi(q)=1$, $\phi(q)=C/q$), it may be concluded that the fraction $(n-3)/2(n-1)$ gives with sufficient accuracy for our present purpose, the portion of the subterminal class lost, (0 if $n=3$, 1/6 if $n=4$, $\frac{1}{4}$ if $n=5$, approaching the typical value $\frac{1}{2}$ as n becomes large).

$$K=\frac{(n-3)}{2(n-1)}f(1/2N) \text{ approximately.}$$

Assume now that a limited number (n') of alleles is possible, of which n are present at any moment. The chance that any given allele, absent from the population, will arise in the next generation by mutation is $2Nv$, if v is the rate of mutational origin of the allele in question. Assuming this to be the same for all of the n' alleles, the total number of missing alleles that will appear in a generation is $2N(n'-n)v$. Equating this to the number of losses, Kn, we must have at equilibrium

$$2N(n'-n)v=\frac{n(n-3)}{2(n-1)}f(1/2N).$$

If u is the rate of mutation *from* one allele to all others, $u=(n'-1)v$

$$u=\frac{n(n-3)(n'-1)}{4N(n-1)(n'-n)}f(1/2N).$$

Figure 1 shows the number of alleles (n) maintained in a population of size N by various mutation rates u, assuming that 25 alleles are possible.

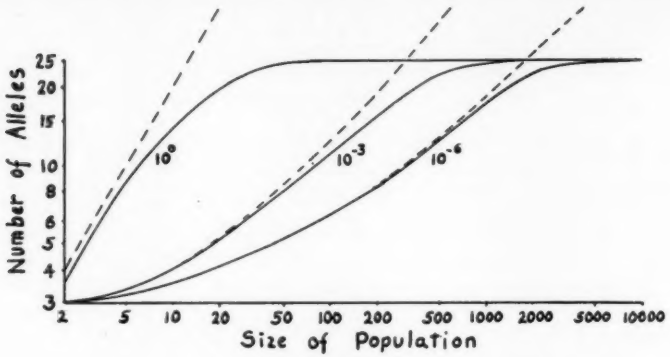


FIGURE 1.—The number of self-sterility alleles maintained in populations of various sizes if 25 different alleles are possible and mutation (or replacement by immigration) is occurring at the rates 10^{-3} or 10^{-6} per generation. The limiting case, complete replacement, is indicated by the curve labelled 10^{0}. The broken lines show the number of alleles if an indefinitely large number are possible.

If new alleles are being introduced into a population to a significant extent by outbreeding rather than by mutation, corresponding equations can be written to express the balance of losses and gains. It is merely necessary to substitute mq_i for v, $m(1-q_i)$ for u and $n_i(=1/q_i)$, the number of alleles in the species as a whole, for n'.

If the number of possible alleles is indefinitely great it is possible that each mutation will represent a wholly novel allele (v indefinitely small). In this case $(n'-n)/(n'-1)$ approaches 1.

$$u = \frac{n(n-3)}{4N(n-1)} f(1/2N).$$

Figure 2 shows the number of alleles (n) maintained in a population of size N by the occurrence of novel mutations at the rate u per generation. Figures 3, 4 and 5 compare the distributions of frequencies in populations of 50 and 500 individuals, at mutation rates of 10^{-2}, 10^{-4}, and 10^{-6}, respectively. The values of the selection index R, the mean number of representatives of an allele ($2N\bar{q}$), the mean number of alleles (n) and the turnover (K) are given in table 1.

COMPARISON WITH EMERSON'S DATA IN OENOTHERA ORGANENSIS

In EMERSON'S data, 34 alleles were demonstrated in a sample of 135 gametes from a population estimated as less than 1000 plants and very

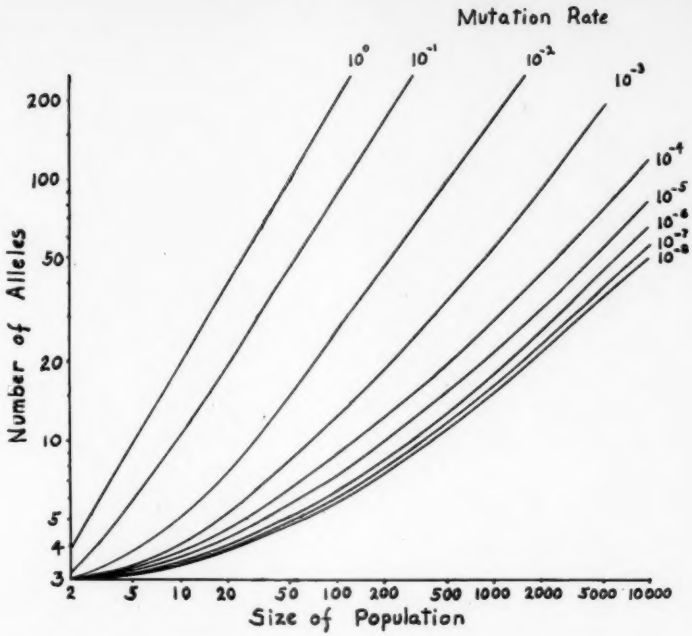


FIGURE 2.—The number of self-sterility alleles maintained in populations of various sizes if the number of possible alleles is indefinitely great and mutation (or replacement by immigration from a very large population) occurs at rates from 10^{0} to 10^{-8}.

TABLE 1

Comparison of populations of 50 and of 500 individuals at three different rates of mutation (or of immigration from an indefinitely large population).

N NO. OF INDIVIDUALS	u MUTATION RATE	R SELECTION INDEX	$2N\bar{q}$ MEAN NO. OF REPRESENTATIVES OF ALLELE	n NO. OF ALLELES	K FACTOR TURNOVER PERCENT
50	.01	.77	6.8	14.8	7
500	.01	.95	10.4	96.0	10
50	.0001	.59	15.4	6.5	0.15
500	.0001	.87	51.3	19.5	0.51
50	.000001	.49	19.5	5.1	0.002
500	.000001	.82	77.7	12.9	0.008

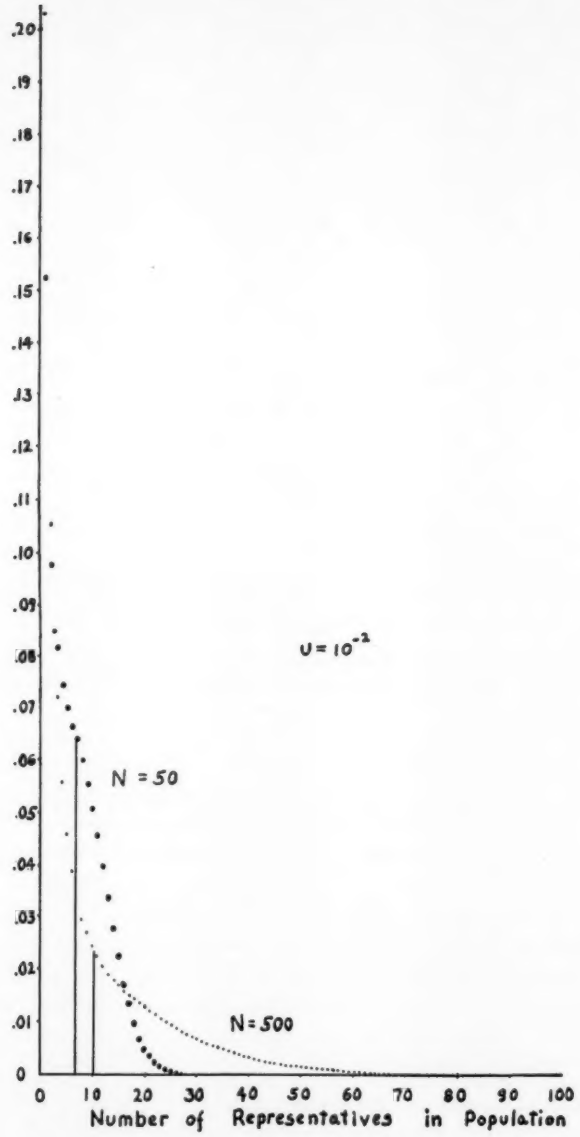
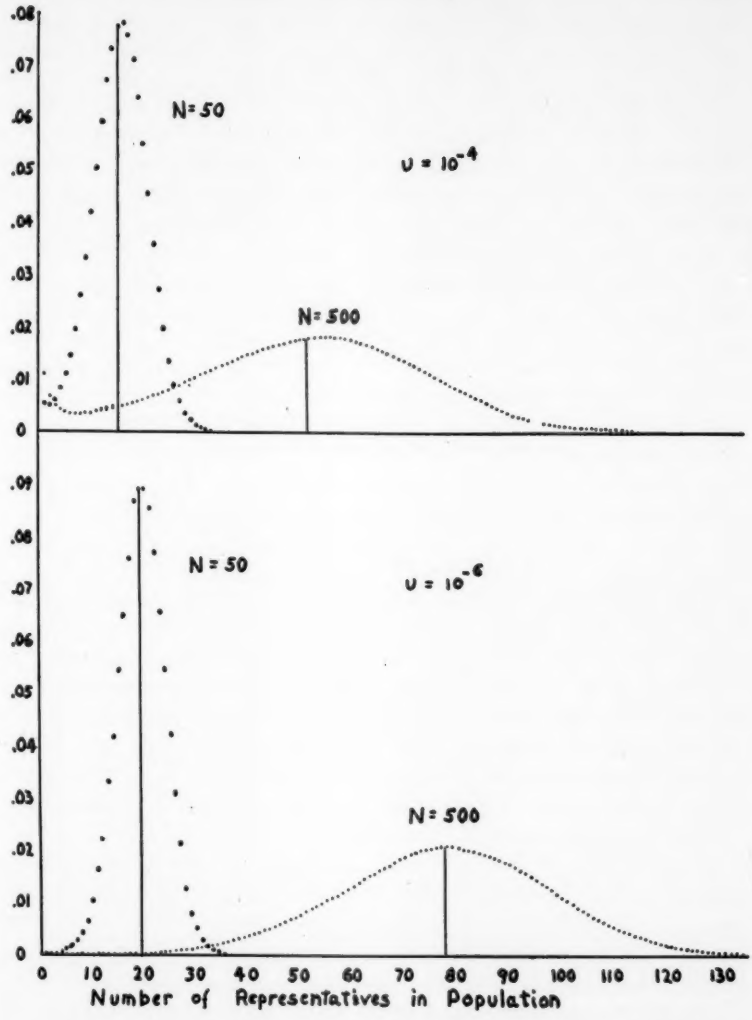


FIGURE 3.—Description under figures 4 and 5.



FIGURES 3 to 5.—The probabilities of different numbers of representatives ($2Nq$) of a self-sterility gene (while present at all) compared in populations of 50 and 500 individuals and with mutation (or replacement by immigration from a very large population) at rates of $u=10^{-3}$ (fig. 3), $u=10^{-4}$ (fig. 4, above) or $u=10^{-6}$ (fig. 5, below). An indefinitely large number of alleles is assumed possible. See table 1.

likely less than 500. It is known that several additional alleles are present in the population and doubtless the number would be considerably increased in a sample of size $2N$. From figure 2, it is apparent that it would require a mutation rate of considerably more than 10^{-3} to maintain such a large number of alleles. Mutation rates of 10^{-5} or less would maintain less than 15 alleles. PROFESSOR EMERSON informs me that in preliminary tests no mutations have been found in 45,000 pollen grains, indicative as far as it goes of a mutation rate decidedly too low to account for the observed number of alleles.

One possible explanation would be that the size of the population is much greater than estimated from the data now at hand or, if not greater now, that it has recently been much greater, with loss of alleles at too slow a rate to have reached equilibrium. Inspection of figure 2 indicates that a population of some 4000 to 5000 is required to account for the probable number of alleles. Another possible explanation would be that some alleles are much more mutable than others. An average rate greater than 10^{-3} seems, however, improbable. Finally, there is the possibility that the large number of alleles is a consequence of local inbreeding. This possibility may be considered by extension of the methods used here.

THE EFFECT OF LOCAL INBREEDING

It is obvious from inspection of figure 2 that subdivision into completely isolated groups would greatly increase the number of alleles carried by the species as a whole, although reducing the number found in a single local collection. In the long run each group would come to have an entirely different set of alleles from every other group. As an isolated population of 50 individuals would maintain five or six alleles at mutation rates of 10^{-6} or 10^{-5}, an assemblage of 10 such groups would maintain 50 or 60 alleles instead of the 13 to 15 expected under random mating. With a finer subdivision, the increase would be still greater.

However, it is clear from EMERSON'S data that there is nothing approaching complete isolation. Of the 34 alleles found in the 1937 collection, seven were found in all three of the most thoroughly studied canyon populations, 14 were found in two of them, leaving 13 which have so far been found in only one. On the other hand, as he points out, there is not completely random interbreeding. There is almost twice as much chance ($114/2699 = .043$) that a second gamete from the same canyon as the one chosen first will have the same allele, as that one from another canyon will agree ($128/5451 = .024$). Even the slightest restriction on random breeding will presumably increase the number of alleles carried by the total population, but whether this increase is an appreciable one where there is as much interbreeding as is indicated in the present case, requires consideration.

We have already considered the distribution of gene frequencies in a local population of size N, receiving the proportion m of its gametes from the species as a whole, with gene frequency q_i. If the species is not indefinitely large, q_i itself is a variable and it is, indeed, the distribution of q_i which we wish ultimately to estimate. However, q_i enters into the formula for $\phi(q)$ in such a way that there is no important error in assuming it constant at the value $\bar{q}_i = 1/n_i$ where n_i is the total number of alleles at which we wish to aim in our calculations. The value of R for the local population may be found as before by trial and interpolation to satisfy the condition of equilibrium.

In considering the species as a whole, it is convenient first to distinguish the proportion $1-2m$ of the fertilizations in which the pollen as well as the ovules come from the local group and the proportion $2m$ in which the pollen is a random sample from the species. In the former, selection pressure depends on the number of alleles in the group and hence on the value of R determined for the group. In the latter it depends on the number of alleles in the whole species. We will consider first the component due to inbreeding.

Assume that there are G subgroups of equal size (N). Let h be the proportion of these that contain the allele S_i. Then q_i/h is the average frequency within these groups. S_i pollen has no chance of success on the $2q_i$ plants that carry S_i. On the $(h-2q_i)$ plants that are of the same subgroups but do not carry S_i, the average frequency of success of S_i pollen to other kinds is in the ratio $q_i/h : R[1-(q_i/h)]$. The frequency of functioning S_i pollen grains is thus

$$\frac{(h-2q_i)q_i/h}{q_i/h + R[1-(q_i/h)]}$$

and the frequency of functioning S_i gametes is

$$\frac{q_i}{2}\left[\frac{(h-q_i)(1+R)}{Rh+q_i(1-R)}\right].$$

It will not do even as a first approximation, to assume that h is constant. If the allele is represented only once in the whole species ($q_i = 1/2N_i$), h is obviously $1/G$. With increasing representation in the species, h may be expected to increase until a point is reached at which the gene is practically always present in all subgroups ($h=1$). As a first approximation, we will assume that h varies linearly with q_i between $q_i = 1/2N_i$ and $h=1$, with such a slope (b) as will yield the required $\bar{q}_i$.

$$h = \frac{1}{G} + b\left(q_i - \frac{1}{2N_i}\right).$$

It will be convenient to write this, $h=a+bq_i$, where $a=(1/G-b/2N_i)$.

In those fertilizations which may be treated as random breeding, the frequency of functioning S_i gametes is

$$\frac{q_i}{2}\,\frac{(1-q_i)(1+R_i)}{[R_i+q_i(1-R_i]}$$

where R_i depends on the number of alleles in the whole species. If the amount of crossbreeding is small, this term is relatively unimportant and a rough approximation will suffice. Indeed if $m=.01$ or less, it makes little difference whether this term is considered, or whether it is omitted altogether, giving full weight to the inbreeding term. If included, we may use $R_i=(n_i-3)/(n_i-1)$, the value if all alleles are equally frequent, as such an approximation. Giving inbreeding and crossbreeding due weight and introducing mutation pressure, the rate of change of gene frequency may be written

$$\Delta q_i=(1-2m)\,\frac{(1+R)}{2}\,q_i\left[\frac{a+q_i(b-1)}{Ra+q_i(Rb+1-R)}\right]$$
$$+\,2m\,\frac{(1+R_i)}{2}\,q_i\left[\frac{1-q_i}{R_i+q_i(1-R_i)}\right]-q_i(1+u).$$

The sampling variance of a subgroup is $q(1-q)/2N$ disregarding crossbreeding. That of the whole species is

$$\sigma^2_{\Delta q_i}=\frac{1}{G^2}\,\Sigma\left(\frac{q(1-q)}{2N}\right),$$

noting that $\Delta q_i=\Sigma\Delta q/G$. A first approximation can be obtained by replacing q by its average value (q_i/h) in those groups (hG in number) that contain it at all.

$$\sigma^2_{\Delta q_i}=\frac{q_i(h-q_i)}{2N_ih}=\frac{q_i[a+q_i(b-1)]}{2N_i(a+bq_i)}\ \text{approximately.}$$

If q_i is small, this differs little from the value $q_i(1-q_i)/2N_i$ in a random breeding population of the same size. The latter may be used for convenience in dealing with the small crossbreeding term in the ratio $\Delta q_i/\sigma^2_{\Delta q_i}$.

Substitution in the general formula for $\phi(q)$ leads to the following expression.

$$\log_{10}\phi(q_i)=\log_{10}(a+bq_i)-\log_{10}q_i$$
$$+\left[\frac{4N_ia(1+u)}{(b-1)^2}-1\right]\log_{10}[a+q_i(b-1)]$$
$$+\,[\log_{10}e]\left[\frac{2N_ib(1+R)(1-2m)}{1+R(b-1)}-\frac{4Nb(1+u)}{b-1}\right]q_i$$

$$+ (1 - 2m)\frac{2N_i a(1 - R^2)}{[1 + R(b - 1)]^2}\log_{10}\left[Ra + q_i[1 + R(b - 1)\right]$$

$$+ 4mN_i\frac{(1 + R_i)}{(1 - R_i)}\log_{10}[R_i + q_i(1 - R_i)].$$

Values of b and u may be found by trial that give the required mean ($\bar{q}_i$) and satisfy the balance between loss and mutation.

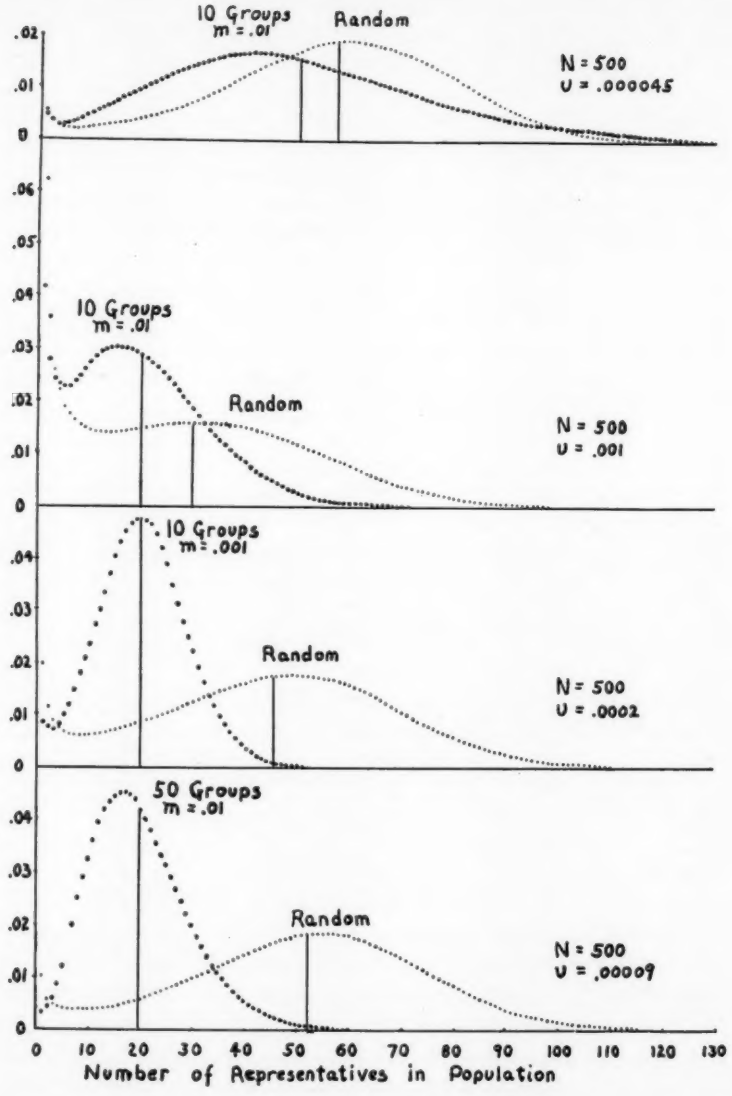
Four cases have been worked through to obtain an indication of the effect of subdivision into partially isolated populations (table 2). In the first case, a total population of 500 was assumed to be divided into 10 equal

TABLE 2

Comparison of subdivided and random breeding populations of 500 individuals and of the partially isolated groups of the former. The columns N, m and u describe the conditions with respect to size of population, immigration and mutation. Columns R and b give two additional constants of the formulae. Column n gives the number of alleles maintained under the given conditions and column K the percentage turnover of alleles per generation.

	N NO. OF INDIVIDUALS	m IMMIGRATION INDEX	u MUTATION RATE	R SELECTION INDEX	b	n NO. OF ALLELES	K FACTOR TURNOVER PERCENT
Group	50	.01	.000045	.732	—	11.6	3.6
Total	500	0	.000045	.732	6.73	20.0	0.23
Random	500	0	.000045	.860	—	17.5	0.25
Group	50	.01	.0010	.755	—	13.4	5.3
Total	500	0	.0010	.755	5.29	50.0	2.0
Random	500	0	.0010	.906	—	33.8	3.0
Group	50	.001	.00020	.658	—	8.2	1.0
Total	500	0	.00020	.658	2.50	50.0	0.43
Random	500	0	.00020	.880	—	22.1	0.90
Group	10	.01	.00009	.425	—	5.3	3.3
Total	500	0	.00009	.425	3.73	50.0	0.18
Random	500	0	.00009	.869	—	19.2	0.45

groups breeding within themselves except for 2 percent foreign pollen. It is required to find the mutation rate that will maintain 20 alleles in the species. By trial it was found that the value of R within groups is .732 and that the mean number of alleles within groups is 11.6 with turnover of 3.6 percent per generation. For the whole population, b was found to be 6.73 and the required mutation rate 4.5×10^{-5}. The rate of turnover of alleles in the species is 0.23 percent. In a completely random breeding population, the same mutation rate would maintain an average of 17.5 alleles with a turnover of 0.25 percent per generation. In this case the



FIGURES 6–9. (See opposite page for description.)

postulated amount of subdivision increases the number of alleles only about 14 percent. The distribution in the subdivided and random breeding populations are compared in figure 6.

With the same amount of subdivision ($G=10$, $N=50$, $m=.01$) it requires a mutation rate of about 10^{-3} to maintain 50 alleles (turnover 2.0 percent per generation). This mutation rate would maintain only about 34 alleles under random mating. The subgroups carry 13.4 alleles on the average (figure 7).

It seemed of interest next to investigate the effect of more complete isolation. It was again assumed that there are 10 subgroups of 50 individuals each but the crossbreeding index was taken as .001 instead of .01. It only requires a mutation rate of 2×10^{-4} to maintain 50 alleles in the species (turn over 0.4 percent), a mutation rate that would maintain 22 alleles under random mating. The subgroups carry only 8.2 alleles on the average (figure 8).

None of the preceding cases approaches a satisfactory interpretation of the situation described by EMERSON in *Oenothera organensis*. To maintain the probable number of alleles in the species requires a higher mutation rate and more extreme isolation than is probable. The subgroups postulated in all of these cases (50 individuals) are roughly comparable to the populations of separate canyons. More alleles have been observed in the separate canyons (average for the three most studied, 20.7) than is permitted by the theory (about eight in the last case).

The only possible interpretation, accepting 500 as an estimate of the total population, assuming mutation rates less than 10^{-4} and assuming equilibrium, seems to be along the line of a finer subdivision. It was accordingly assumed next that the species is divided into 50 local groups of 10 individuals and that these groups breed within themselves except for 2 percent foreign pollen. With this degree of subdivision, it requires a mutation rate of 9×10^{-5} to maintain 50 alleles in the species (turnover 0.18 percent). This rate would maintain only about 19 alleles under random

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EXPLANATION OF FIGURES 6–9

FIGURES 6–9.—(from top to bottom). The probabilities of different numbers of representatives of a self-sterility allele (while present at all) in populations of 500 individuals, compared in the cases of random breeding and of subdivision into partially isolated groups.

FIGURE 6.—Ten groups, $m=.01$, 20 alleles in the subdivided population. The implied mutation rate (4.5×10^{-5}) would maintain 17.5 alleles under random mating.

FIGURE 7.—Ten groups, $m=.01$, 50 alleles in the subdivided population. The implied mutation rate (10^{-3}) would maintain 34 alleles under random mating.

FIGURE 8.—Ten groups, $m=.001$, 50 alleles in the subdivided population. The implied mutation rate (2×10^{-4}) would maintain 22 alleles under random mating.

FIGURE 9.—Fifty groups, $m=.01$, 50 alleles in the subdivided population. The implied mutation rate (9×10^{-5}) would maintain 19 alleles under random mating.

mating (figure 9). The groups of 10 plants should carry an average of only 5.3 alleles; but a canyon containing several such groups, would, of course, maintain several times this number.

This last case obviously comes closest to a satisfactory interpretation and even closer inbreeding would be still more satisfactory. It appears that if plants are pollinated in some 98 percent or more of the cases by their immediate neighbors and only 2 percent or less by a random sample of pollen from the species as a whole, it would be possible for a species of only 500 individuals to maintain 40 or 50 alleles by mutation rates of the order of 10^{-5} or 10^{-6} per generation.

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A STUDY OF INTERSEXUALITY IN DROSOPHILA VIRILIS[1]

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I. GENETICS OF INTERSEXUALITY

IN THIS paper a detailed account of our study of intersexuality in *Drosophila virilis* will be given. The progress of this work has been reported elsewhere (LEBEDEFF 1934a and b, 1938a and b). These intersexes are the result of mutative changes within the normal diploid system, and they differ from intersexes in other species genetically, morphologically and embryologically. Intersexes in Lymantria usually appear from interracial crosses, presumably due to an unbalanced condition between the female and male determining tendencies (GOLDSCHMIDT 1934). Intersexes in Lymantria also have been reported from intraracial crosses (KOSMINSKY 1930). In *D. melanogaster* intersexes appear from the cross between triploid females and normal diploid males (BRIDGES 1932). The chromosomal constitution of these intersexes was found to be intermediate between that of the normal female and the normal male. Several intersexes of the same nature have been obtained from a cross between triploid *D. melanogaster* females and normal *D. simulans* males (SCHULTZ and DOBZHANKSY 1933). A single autosomal gene was found to be responsible for intersexuality in *D. simulans* (STURTEVANT 1921).

The intersexes described here are the result of the action of a single autosomal gene, called intersex (*ix*). The gene was called *ix* soon after it had been discovered, and before its nature had been studied. Since then it has been renamed ix^m to denote its true nature as a gene for maleness. These intersexes were observed first in 1933 in a short vein (*sv*) stock which originated in 1930–31 in one of the cultures received in Cold Spring Harbor from PROFESSOR T. KOMAI, Kyoto, Japan. By inbreeding normal flies from the stock it was observed that some cultures produced intersexes in abundance, while in other cultures intersexes were less numerous. Altogether 40 cultures of the first type were obtained, these producing 2088 males, 1191 females and 962 intersexes. The number of normal males, females, and intersexes approached that of a 2:1:1 ratio; in other words, in these sets of cultures one-half of the female population became intersexual. The 41 cultures in which intersexes appeared less frequently, pro-

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duced 1993 males, 1516 females and 489 intersexes, thus approaching a 4:3:1 ratio of males, females, and intersexes. In this case one-quarter of the female population became intersexual. These data indicate that females homozygous for the ix^m gene become intersexes, that this gene has no effect on males, and that ix^m/ix^m males are fertile. The slight departure from the 2:1:1 and 4:3:1 ratios is explained by assuming that at the beginning of the work the extreme female- and male-like intersexes were classified as females and males. The equal number of 2:1:1 and 4:3:1 segregating cultures is due to the fact that some selection was practiced and only cultures giving good segregation were recorded and propagated.

Many breeding data were accumulated during the work with the intersexes. The original stock was subjected to selection for the various morphological types. Three distinct morphological types of intersexes were isolated: female-like, male-like, and extremely male-like called respectively types 1, 3, and 4. The stock cultures of each line were kept and counts recorded for some time. The summaries of all these cultures are given in tables 1 and 2. Altogether 269 cultures producing different morphological

TABLE 1

Summary of cultures segregating in 2:1:1 ratio for different morphological types of intersexes.

TYPE OF INTERSEX	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES
Line 1	43	1412	790	669
Line 3	55	2127	1023	997
Line 4	76	2539	1354	1222
	174	6078	3167	2888

TABLE 2

Summary of cultures segregating in 4:3:1 ratio for different morphological types of intersexes.

TYPE OF INTERSEX	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES
Line 1	51	1543	1254	411
Line 3	15	479	355	113
Line 4	29	972	706	231
	95	2994	2315	755

types of intersexes were counted. Among these, 174 cultures segregated in a 2:1:1: ratio, giving 6078 males, 3167 females and 2888 intersexes. The other 95 cultures segregated in a 4:3:1 ratio, producing 2994 males, 2315 females and 735 intersexes.

Linkage. A set of experiments was performed to determine the chromosome in which the ix^m gene is located. The normal males[2] from stocks throwing intersexes were crossed with virgin females from stocks which carried the following dominant genes: clipped (*Cl*), in the fourth chromosome (lethal when homozygous); garnet (*G*), in the third chromosome; and rounded (*R*), in the second chromosome. The F_1 males from such crosses were outcrossed to virgin females from the intersex-throwing stocks. From the $+/+\ ix^m/+$ female and $Cl/+\ ix^m/+$ male cross, three cultures were obtained giving altogether 130 flies; of these 63 were males, 50 females and 17 intersexes. Of these 17 intersexes, 9 were clipped and 8 were of the wild type, which indicates that the ix^m gene is inherited independently of the fourth chromosome genes.

For the linkage test with the second and the third chromosomes, a compound rounded garnet stock was used. Virgin females from this stock were crossed to normal males from intersex producing stock. From the cross of F_1 males to $ix^m/+$ females seven cultures were obtained which segregated for intersexes. They gave 419 males, 392 females and 59 intersexes. Twenty-seven of these latter were rounded, 32 were of the wild type, and none of the rounded garnet and garnet types. This indicates that the ix^m gene is independent of *R*, the gene of the second chromosome, and is linked with the garnet (*G*) gene of the third chromosome.

From the reciprocal cross $G\ +/+\ ix^m$ females with $+\ ix^m/+\ ix^m$ or $+\ ix^m/+\ +$ males (the rounded gene can now be disregarded), a number of cultures were obtained in which the ratio of females to intersexes was modified from the expected 1:1 and 3:1 into 3:1 and 7:1 respectively by the presence in the garnet stock of a dominant suppressor of the ix^m gene. Though these data could be used for calculation of the linkage between *G* and ix^m, garnet females were repeatedly mated to the wild type males from this cross in order to eliminate the ix^m suppressor. It was soon observed that, from the cross between $G\ +/+\ ix^m$ females and $+\ ix^m/+\ ix^m$ or $+\ +/+\ ix^m$ males, cultures appeared giving 2:1:1 and 4:3:1 segregation for males, females and intersexes. In other words, the suppressor was eliminated from the stock. The 2:1:1 segregating cultures were recorded and have been used in connection with the linkage studies. Altogether sixteen cultures have been obtained (table 3). In these cases the progeny is the result of a backcross for both *G* and ix^m, and therefore both female and intersexual populations can be used to compute the linkage value between the *G* and ix^m genes. Disregarding the males, the population consisted of 332 garnet

[2] For these and for most crosses in this experiment flies were used preferably from cultures showing a 2:1:1 segregation. Males were used in preference to females, since one-half of the male population from such cultures was homozygous for the ix^m gene, the other half being heterozygous ($ix^m/+$). All of the females from such cultures were heterozygous ($ix^m/+$), the intersexes being homozygous (ix^m/ix^m).

females, 31 wild-type females, 19 garnet intersexes, and 278 wild-type intersexes. The percent of crossing over between *G* and *ix*^{m} is equal to $(50/660)\times 100 = 7.58$.

TABLE 3

Linkage study. The result of the cross of G +/+ ix^m *females and + ix*^{m}*/+ ix*^{m} *males.*

NUMBER OF CULTURES	MALES		FEMALES		INTERSEXES	
	G	+	*G*	+	*G*	+
16	370	366	332	31	19	278

Three point test. In order to find the exact locus of the *ix*^{m} gene, a three point linkage test was conducted. A short vein (*sv*) stock[3] carrying the *ix*^{m} gene was made, and the *sv* females from the stock throwing intersexes were crossed to garnet males.[4] The F_1 generation in this, as well as in the other F_1 crosses, consisted of normal males and females. Then F_1 females (+ + *G*/*sv ix*^{m} +) were backcrossed to *sv* males from the cultures giving 2:1:1 segregation for males, females, and intersexes. The results of these crosses are given in table 4.

TABLE 4

The three-point test. Summary of the crosses between + + G/sv ix^m *+ females and sv ix*^{m} *+/sv ix*^{m} *+ or sv ix*^{m}*+/sv++ males. Females and intersexes only were classified in regard to phenotype.*

RATIO	NO. OF CULTURES	MALES	FEMALES	INTERSEXES	0			1		2		1–2	
					sv ix^m	*G*	*sv G*	*ix*^{m}	*sv ix*^{m} *G*	+	*sv*	*ix*^{m} *G*
4: 3:1	2	80	80	27	19	45	5	6	2	5	23	—
8: 7:1	23	894	763	112	98	346	52	4	9	45	320	1
16:15:1	16	707	590	35	29	273	40	5	1	33	247	—
32:31:1	15	596	556	15	11	234	47	4	—	37	233	—
	56	2277	1989	189	157	898	144	19	12	120	823	1

There are certain peculiarities involved in the results of this cross which need some explanation. First, out of 56 cultures obtained, not a single one showed a 2:1:1 segregation in spite of the fact that approximately one-half of the males used undoubtedly were homozygous for *ix*^{m}. Instead, the intersexual population was greatly deficient. The detailed discussion of this and the other cases of modification of the intersexual ratios will be presented in connection with the study of the modifying factors. It is evident, that in these data only the intersexual population can be considered

[3] Short vein is a third chromosome recessive gene.

[4] The garnet males were taken from *cn G* stock, and not from *RG* stock used for the two point test.

for calculation of linkage relationships between the *sv*, *ix*^{m} and *G* genes. Since intersexes were found but once in each of the possible classes, namely, non-crossover, single and double crossovers, the data are unquestionably reliable. The only disadvantage in using only one representative of each possible class for linkage purposes is that the number of observations is reduced. The percent of crossing over between *sv*$-$*ix*^{m} is $(19+1)/189 \times 100 = 10.6$; between *ix*$^m-G$, $(12+1)/189 \times 100 = 6.88$; and between *sv*$-G$ it is $(19+12)/189 \times 100 = 16.4$. The order of the genes is therefore *sv ix G*. The data in regard to *sv*$-G$ genes are in fairly good agreement with those of the standard third chromosome map (CHINO 1936). The following is a map of the left end of the third chromosome:

rl	*G*	*ix*^{m}	*sv*
0	8	15	25

Sex-linked genes and intersexes

In order to demonstrate that intersexes of all morphological types (and they vary from female-like to male-like) are not gynandromorphs, which they often resemble (DOBZHANSKY 1931), and in order to prove that they are females (XX constitution), sex-linked genes were introduced into the intersex-producing stocks. Yellow, vermilion, forked, miniature, and singed stocks were obtained which segregated for intersexes. From the crosses between females from these stocks and the wild-type males from the stocks throwing intersexes were obtained 934 males, which carried one or another of the above mentioned sex-linked factors, 800 wild-type females, and 397 wild-type intersexes of all morphological types (table 5). The slight defi-

TABLE 5

Sex linked genes and intersexes. Result of crosses between females homozygous for sex linked genes and heterozygous for ix^m*, and wild type males from intersex-throwing stocks.*

FEMALE PARENT	NUMBER OF CULTURES	MALES	WILD TYPE FEMALES	WILD TYPE INTERSEXES
Forked	10	267 *f*	321	131
Vermilion singed	13	382 *v si*	314	164
Vermilion miniature	4	237 *v mi*	127	83
Yellow	2	48 *y*	38	19
	80	934	800	397

ciency in males is probably due to sex-linked simi-lethals, and the imperfect ratio of females to intersexes is due to the fact that some cultures segregated into 3:1 and the others into 1:1 ratios as to the number of females to intersexes. The fact that intersexes from these crosses were of the wild type like the normal females indicates that they are of XX con-

stitution. This is true for all morphological types, including extreme female- and male-like types.

II. MORPHOLOGY OF INTERSEXES

Morphologically intersexes exhibit a high degree of variability in respect of each separate feature of sexual organs, which may be like those of females, males, or both, and may be either reduced or absent. However, for genetical studies the intersexes were grouped into four morphological classes: female-like, hermaphrodites, male-like, and extremely male-like.

The female-like (type 1) intersexes differ from true females externally in that the anal tubercle, instead of having two simple dorso-ventral valves, shows valves divided laterally into four half valves. Often the lateral cleft is incomplete, so that the anal tubercle is composed of three parts. The vaginal plates of these specimens are often slightly displaced from their symmetrical lateral position. This change in the form of the anal tubercle is often accompanied or preceded by the presence of an outgrowth, which is a rudiment of the male external genitalia. It is found near the caudal end of the abdomen, between the anal and vaginal plates (figure 1, A–C). Internally these intersexes differ from the true females in that one, or both, of the ovaries is rudimentary and contains no growing eggs. Rarely ovaries are overdeveloped and contain growing eggs, which are sterile (Plate I, 2 and 4). Often gonads of these intersexes develop into ovotestes, which are usually attached by their ovarian part to the oviduct. The testicular part may be either attached or unattached to the rudimentary seminal vesicles (Plate I, 3 and 6).

In hermaphrodites (type 2 intersex) the male reproductive organs, both external and internal, are far more developed than in the previous type. The male external genitalia in hermaphrodites are developed to such an extent that the claspers and a penis are present. However, they are often incompletely formed. The female genitalia are still more displaced from their lateral position. The anal tubercle in these specimens usually has characteristics of both sexes, having both dorso-ventral and lateral clefts. Often the dorso-ventral cleft is incomplete or entirely absent, so that the anal tubercle is either composed of three parts, or assumes entirely a male-like aspect (figure 1D; figure 2A). The male sexual ducts in hermaphrodites are developed to various degrees. In the weak hermaphrodites seminal vesicles, and often paragonia are the only organs which are completely formed. In the more developed hermaphrodites the male sexual ducts are well developed, so that paragonia, sperm pump and vas deferens are easily seen upon dissection (Plate I, 5, 7 and 8; Plate II, 1). The gonads of hermaphrodites are ovotestes, and often are attached to both the female and the male sexual ducts.

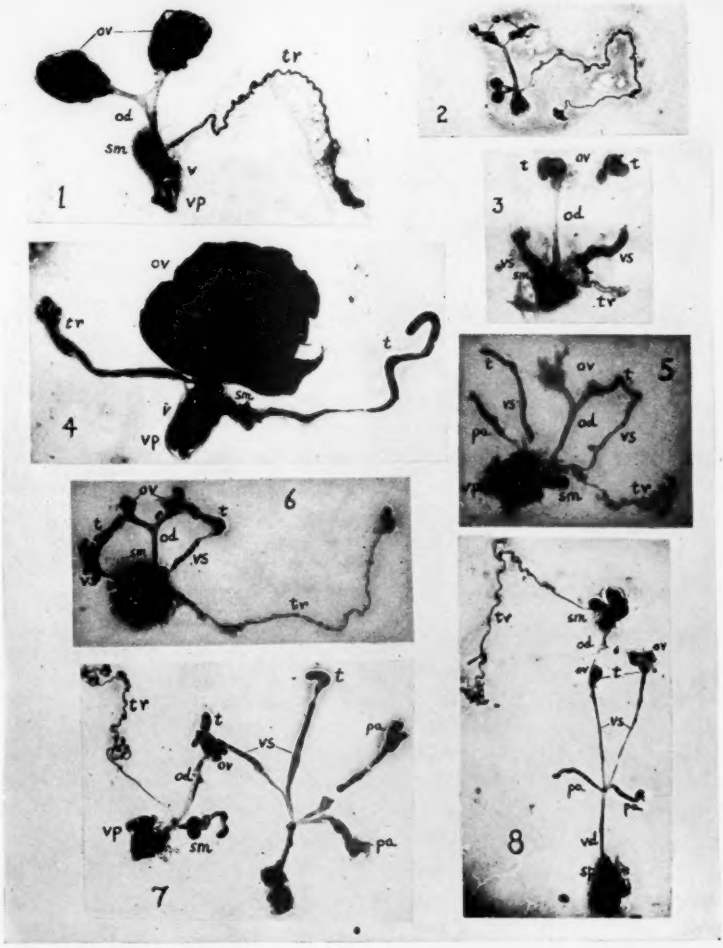

PLATE I.—Internal reproductive organs of: 1, normal female; 2 and 4, most female-like intersex; 3, 5 and 6, weak hermaphrodite; 7–8, complete hermaphrodite. Microphotographs, magnification about 22–25.

od—oviduct; pa—paragonia; sm—spermathecae; sp—sperm pump; t—testes; tr—tubular receptacle; v—vagina; vd—vas deferens; vp—vaginal plates; vs—seminal vesicles.

The genitalia of normal males, as seen posteriorly, are rotated counter clockwise through 360°, so that the duct (ductus ejaculatorius) loops once about the intestines. This can easily be observed in the hermaphrodites. As soon as the male genitalia in these intersexes have reached a certain degree of development they begin to rotate around the duct to a considerable degree. During this process they are often drawn into the abdomen together with the female genital plates and the anal tubercle. In these cases specimens seem to lack any external genitalia, and often an anal

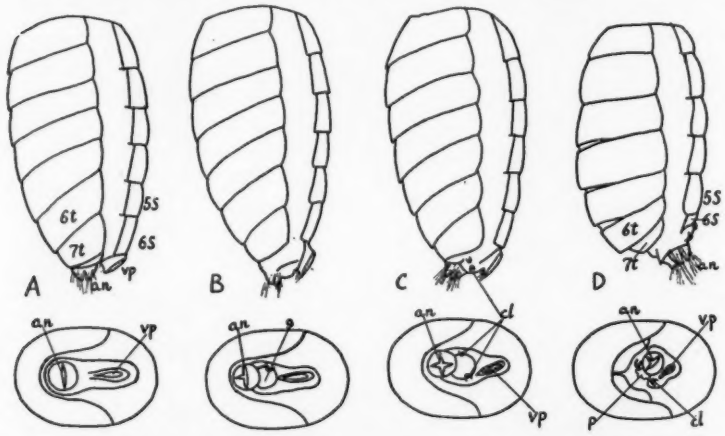

FIGURE 1.—Abdomen and external reproductive organs of: A, normal female; B–C, female-like intersex (type 1); D, weak hermaphrodite.
an—anal opening; cl—claspers; g—rudiments of male genitalia; mg—male genitalia; p—penis; vp—vaginal plates; 4S–6S—fourth–sixth sternites; 5t–7t—fifth–seventh tergites.

opening. However, dissection reveals their presence. In cases where the anal tubercle is involved, this process is frequently fatal to the individual as the feces are liberated into the body cavity, there being no opening to the exterior.

There is apparently some correlation between the degree of the development of external and internal sexual organs of the male in these hermaphrodites. However, instances were recorded when the male internal sexual organs were quite well developed, while the male external genitalia were not yet completely formed. The appearance and the development of the male system in these intersexes usually do not interfere with the complete development of the female system. However, in many cases it was observed that in intersexes of these types the female system begins to disappear before the male sexual organs are fully developed.

The complete development of the male system is accomplished in the

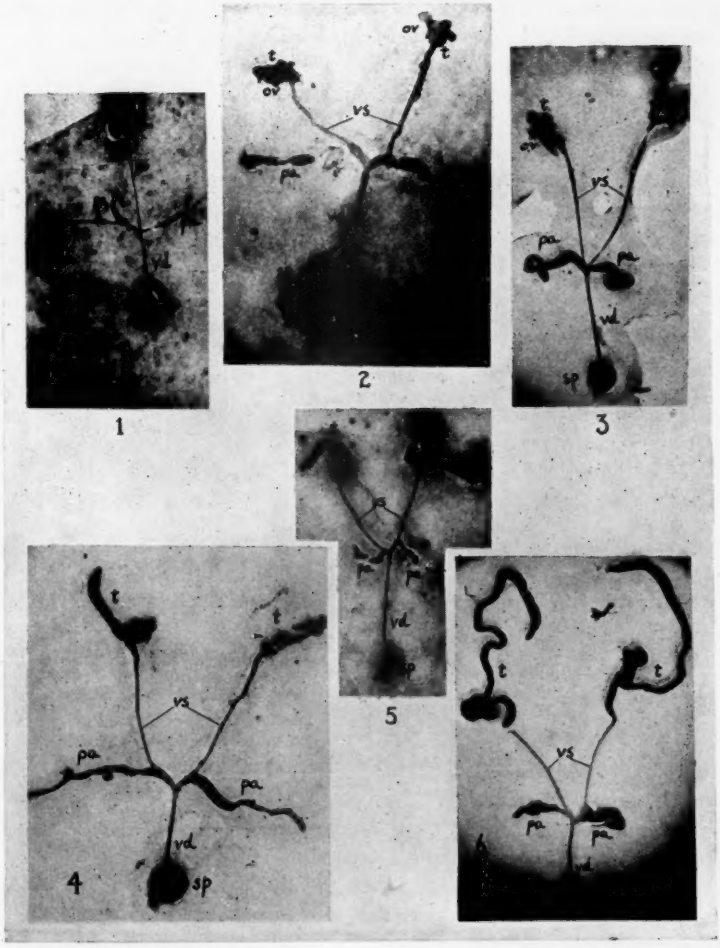

PLATE II.—Internal reproductive organs of: 1, hermaphrodite; 2–3, weak-male (type 3) intersex; 4–5, male-like intersex; 6, normal male. Magnification and lettering same as in the Plate I.

next two types of intersexes. It is usually accompanied by the gradual disappearance of the female sexual organs, leading to an almost complete sex reversal. In the type 3 intersexes both male external and internal sexual organs are almost completely formed. The male external genitalia, however, are located in an unsymmetrical position (figure 2, B). The gonads in these intersexes are ovotestes, which are usually attached to the male sexual ducts only; the oviduct lies free. In most cases the detachment of the oviduct from the ovotestis is accompanied by the reduction or the complete disappearance of the oviduct and often of the tubular receptacle (Plate II, 2–3). The last internal female organ (excluding the ovary) to

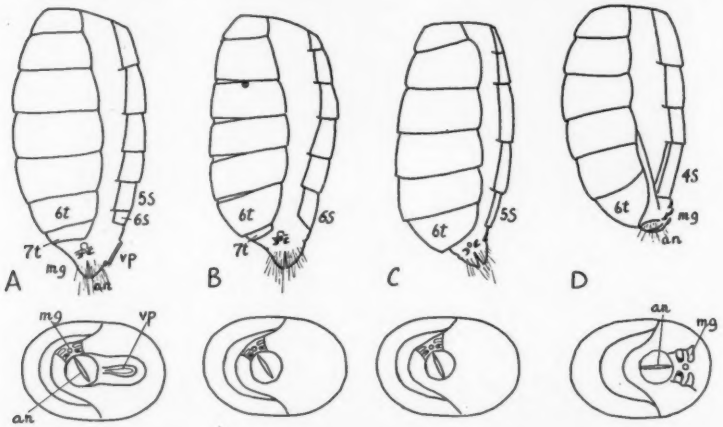

FIGURE 2.—Abdomen and external reproductive organs of: A, hermaphrodite (type 2); B, weak male-like (type 3) intersex; C-D, male-like intersex (type 4); D also of a normal male. Lettering as in the figure 1.

disappear is the spermatheca. In all the other types of intersexes the shape of the abdomen is that of a normal female. In this type, however, the abdominal characteristics for the first time show some signs of change; the seventh and the sixth tergites show some tendency to fuse.

The type 4 intersexes resemble true males externally and internally. The male external genitalia reach symmetrical or nearly symmetrical lateral positions. The number of abdominal segments (tergites and sternites) is gradually reduced to the number found in normal males (figure 2, C–D). These changes are accompanied by the disappearance of the ovarian part of the gonads and the development of testes (Plate II, 4–5). Often ovarian tissue can still be found attached to one or both of the testes in these intersexes. The converted males do not have sperms in their testes and are sterile, as are all the other types of intersexes.

To complete the list of various types of intersexes found in this study, some odd types should be mentioned. Among sex intergrades of the hermaphrodite types several cases were observed in which the number of spermathecae was increased from the usual two to three and four. Sometimes the number remained normal, but the shape was slightly altered from the oblique to slightly flattened, the size being also reduced in some cases. In several instances it was found that one of the testes, together with the seminal vesicle and one paragonium, was missing from the male part of the hermaphrodite. Several instances were recorded of converted males having only one testis and one set of ducts. In one case a converted male was found with two perfectly symmetrical sets of male external genitalia, to each of which one testis, together with a seminal vesicle and a single paragonium was attached. Finally several individuals resembling normal males were found which did not show any sign of a reproductive duct system nor any external genitalia, but which had in their body cavity two red ellipsoidal testes.

From this brief description of the morphology of intersexes it is evident that there is at once a striking similarity and a striking dissimilarity between intersexes of *D. virilis* and those of Lymantria and *D. melanogaster*. GOLDSCHMIDT found that Lymantria intersexes possess a mixture of male and female characteristics, and that a condition of intersexuality did not effect all the sexual characteristics equally in a given individual. Thus some organs of an intersex were normal male or female, while the others were intersexual. Further investigations have demonstrated that the mixing of the male and female characteristics in a given intersex is not accidental, but is a result of a very definite process in the embryonic development. The intersex develops for a time as the sex indicated by the genotype; then from a certain point, the turning point, it continues to develop under the control of the other sex. As a result, some of the characteristics of the initial sex of the individual are transformed or replaced by corresponding sexual characteristics of the other sex. The degree of intersexuality is determined by the time of occurrence of the turning point. DOBZHANSKY and BRIDGES (1928) concluded that the same principle holds for intersexuality in *D. melanogaster*. They found that there is a definite relation between the stability of a character in the intersex and the time of development of this character in normal sexes.

The conclusion that the principle of the turning point provides the mechanism for the formation of *D. virilis* intersexes is based on genetical, cytological, and morphological studies of intersexes. The genetical and cytological evidences indicate that intersexes of all morphological types, including converted males, are of 2X:2A constitution, or zygotic females. Morphological studies of *D. virilis* intersexes reveal that the first male

characteristic to appear in intersexes is the changed structure of the anal tubercle. This is followed by the appearance and development of the male sexual duct system and penis, together with the formation of the genital arch and the fusion of the sixth and seventh tergites. In other words, there is a definite relation between the order of the appearance of the male sexual characteristics in intersexes and the time they originate and differentiate in the pupal development. For the female characteristics the situation is exactly reversed. The late developing female characteristics, such as the organs of the external genitalia, tend to be the first to disappear in the intersexes. The early developing female characteristics, such as the vagina and tubular receptacle, are the last to disappear in the intersexes. Therefore, it can be assumed that *D. virilis* intersexes develop as females up to a certain point, after which they start to develop in a male direction.

However, morphological studies of these intersexes indicate that while the turning point is responsible for the intersexuality here, the embryology of those sexual organs which had not completed their development at the time of the occurrence of the turning point must be different in intersexes of *D. virilis* from that in Lymantria and *D. melanogaster* intersexes. In the case of *D. virilis* intersexes, all sexual organs, with the exception of the secondary ones, which have not completed their development at the time of reversal, do so after the occurrence of the turning point. The turning point does not interfere with the development of most of the sexual organs of the initial sex (female in the case of *D. virilis*) once the imaginal discs of these organs have been laid down, even though they have not sufficiently differentiated. However, the occurrence of the reversal reaction stimulates the development of the corresponding sexual organs of the other sex. The male organs develop from fresh outpushings (or else from new imaginal discs). As a result, the two systems, the initial female and the additional male, develop side by side in the same individual, resulting in the development of hermaphrodites of various degrees.

All these considerations on the nature of intersexuality in *D. virilis* have been based on genetical, cytological and morphological studies of intersexes. The embryological development of sexual organs in intersexes can be interpreted by the morphological studies of the adult intersexes, provided the embryology of the normal male and female is well known. The development of sexual organs in normal sexes of *D. melanogaster* was studied by DOBZHANSKY and BRIDGES (1928). The conclusions as to the nature of intersexuality in *D. virilis* have been based on their work, the assumption being that the sequence of appearance and development of imaginal discs of the sexual organs are basically the same in both species.

III. THE DEVELOPMENT OF THE GONADS IN INTERSEXES

Materials and methods

The intersexes from our lines 1, 3 and 4 were subjected to analysis. From three to five pairs of flies, preferably from cultures the male parents of which were homozygous for *ix*^{m}, were placed in clean half-pint milk bottles. Strips of blotting paper smeared with culture media and seeded with yeast suspension were placed on regular slide glasses and slipped into these culture bottles. Cultures were kept at 22–25°C. Every twelve hours the blotting paper with the media and the deposited eggs was removed and placed in fresh clean bottles, while new medium was introduced into the bottles containing the flies. The eggs were examined every six or twelve hours, and newly hatched larvae were removed to the regular culture bottles. Under these conditions the egg period lasted about 1½ days, larval period 6½–7 days and pupal period 6½–7 days. When the larvae and the pupae reached a certain age, they were removed from the culture and their sex determined. In most of the cases the gonads were dissected and then fixed. In several instances the tissues were fixed in situ with less satisfactory results, as has been the experience of all Drosophila cytologists. A strong Flemming solution was used for fixation and iron-alum haemataxylin for staining.

Gonads in intersexes of types 2 and 3

Most of our studies on the development of gonads of intersexes were made with these two types. There are about sixty slides available of gonads of these intersexes, from about four day old larvae up to the adult stage. Since the development of the gonads in these two types of intersexes is essentially similar, it will be considered together.

During the larval stages, the gonads of these intersexes resemble both in size and shape the gonads of normal male larvae of the same age (Plate III, 11–12, 16–17). The germinal cells, however, are distinctly female-like in aspect; they are oogonia and oocytes in various stages of development. These cells, however, are considerably larger than oogonia and oocytes found in the normal ovaries, as they are in the process of transformation into spermatocyte-like cells. The young unencysted oogonia are found in the most distal part of these gonads. Next to them are located cysts with the young oocytes, while those with oocytes in more advanced stages are found in the central and the proximal parts of the gonads. Often cysts with young oocytes are also found among those with oocytes in more advanced stages of development. There are usually 16 oocytes in the cysts. In several instances as many as 32 or more oocytes were found to be in the same cyst.

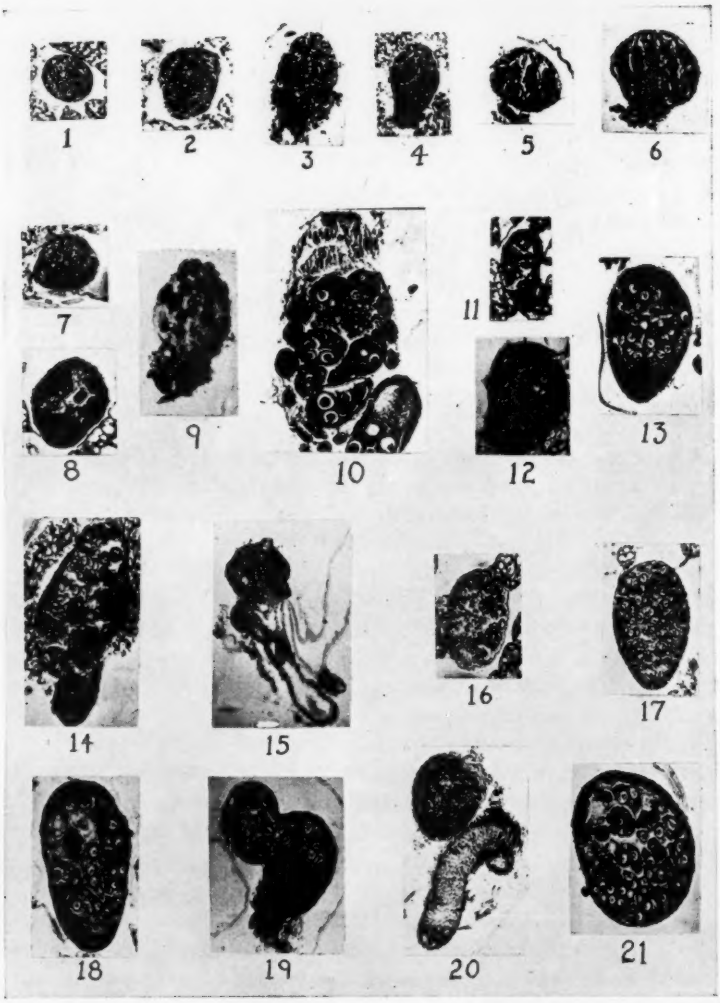

PLATE III.—Gonads of normal females; 1–3, larval stage; 4–6, pupal stage. Gonads of female-like intersex (type 1); 7–8, pupal stage; 9–10, adult stage. Gonads of hermaphrodites (type 2); 11–12, larval stage; 13–14, pupal stage; 15—adult stage. Gonads of male (type 3) intersex; 16–17, larval stage; 18–19, pupal stage; 20, ovotestis of the adult; 21, ovarian part of the ovotestis. Microphotographs from permanent preparations, magnification about 125 diameters.

Beginning with the late larval stage the gonads of these intersexes become smaller than those of the normal male of the same age, and therefore can be distinguished without difficulty from the gonads of normal males and females (Plate III, 13–14 and 18–19). By this time some of the oocytes in the most proximal part of the gonads have increased considerably in size and have undergone a transformation into spermatocyte-like cells. Some of these transformed spermatocytes show signs of disintegration.

The late pupal stage of the gonads of these intersexes is the most difficult to study, as the gonads appear as having an indefinite shape and size, while in the stages preceding they were of considerable size and of a definite shape. Our preparations of the gonads in this stage are not very satisfactory. There is a considerable amount of pycnosis in them, and a number of germinal cells were found which seemed to be outside of the boundaries of the gonads. However, these preparations, when taken together with those of the gonads of freshly hatched intersexes of these types, suggest that during the late pupal stage the ovary gives rise to a testis, both organs then remaining attached to each other as an ovotestis. The process of transformation of these gonads is not yet clear. There is an indication that in some cases this might be accomplished by a simple budding (Plate III, 14, 19). The evidence from dissections of the gonads of these intersexes in the larval and pupal stages suggests that it is unlikely that the ovotestis starts to develop as two distinct organs. In no case was more than one pair of gonads found in the dissected larvae and the young pupae of these intersexes.

The adult intersexes emerge with two sets of gonads (Plate III, 15 and 20–21). The ovarian part is a well defined rounded body, especially in the type 3 intersex, and filled with oogonia and oocytes in the various stages of development. The testicular part is tubular in shape and is mostly filled with disintegrating cells. There is considerable variation in the degree of development of the ovotestes, especially in type 2 intersexes. The peritoneal sheath of the ovarian part of the ovotestis remains colorless, while that of the testicular part becomes red.

Gonads in the male-like (type 4) intersexes

The earlier stages of the development of gonads in these intersexes are essentially similar to those of the type 3 intersexes, as can be seen from the limited number of preparations (Plate IV, 1–4). The gonads of the male-like intersexes, however, are larger, reaching almost the size of the gonads of normal males. The other striking characteristic of the gonads of these intersexes at this stage is the absence of the giant spermatocyte-like cells. Apparently the process of transformation of the oocytes into spermatocyte-like cells in this case is much retarded.

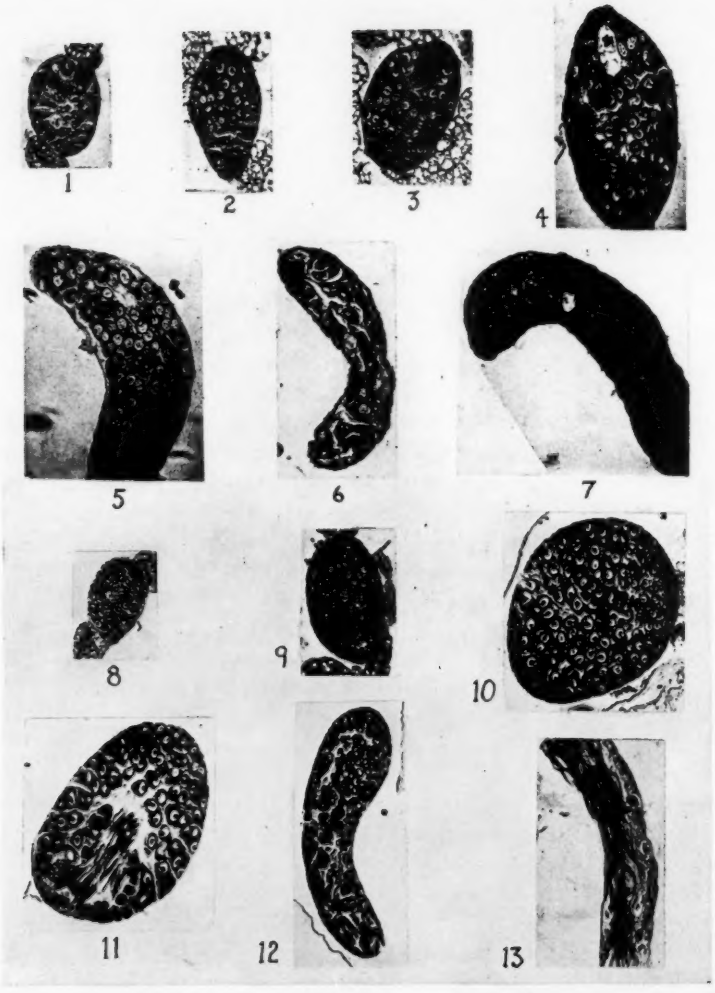

PLATE IV.—Gonads of male-like (type 4) intersex; 1–2, larval stage; 3–5, pupal stage; 6–7; of the adult intersex (only part of the testis is shown). Gonads of normal male; 8–9, larval stage, 10–12, pupal stage; 13, of the adult (only part of the testis is shown). Magnification about 125 diameters.

The gonads of these intersexes continue to grow at almost the same rate as the normal testes. At the late pupal stage they elongate and assume a tubular form. Only at this time, or shortly before, can the giant cells be found in them (Plate IV, 5). The testes of the adult male-like intersex are mostly filled with spermatocyte-like cells, some of which are disintegrating. In several instances these testes were filled with small, spermatogonia-like cells (Plate IV, 6–7).

Gonads in type 1, female-like intersexes

An account of the development of gonads in intersexes would not be complete without some reference to the development of gonads in the female-like intersexes. At present our series of sections of the gonads of this type of intersex is far from complete. Considerable material is available in the sections of gonads of adult intersexes of this type (Plate III, 9–10). Also, several sections of these gonads in the larval stage were obtained (Plate III, 7–8). As may be seen from this material, the gonads of the intersexes of the female type during the larval stage approach in size and cell content the gonads of normal females of the same age. There is considerable variation in the condition of the gonads in the adult intersexes. Some of the gonads of the adult intersexes of this type are in a rather rudimentary state of development, comparable with the gonads of normal females at larval or pupal periods. They are filled with very small oogonia and oocytes in various stages of development. These cells are encysted, but there is no evidence of the formation of ovarioles. On the other hand, some of the gonads of the adult intersexes of the female type are quite well developed, containing ovarioles at different stages of development, and sometimes even a few growing eggs. The nuclear contents of these gonads have not been studied, and it is not known whether or not meiosis has occurred in the growing eggs.

The study of the development of gonads in various morphological types of intersexes indicates that in all of these morphological types the gonads start their development as ovaries. In the female type of intersex the ovaries continue their development, though it is retarded in various degrees. In the hermaphrodites, a part of the female germ cells in the ovaries are transformed into spermatocyte-like cells, while the ovary gives rise to a testis-like structure in which are found some of the oocytes now transformed into spermatocytes. The transformation of female germ cells into male-like ones is often accompanied by the disintegration of these cells. In the male type of intersexes the ovaries are gradually transformed into testes-like organs, while their cell contents undergo the process of transformation into spermatocyte-like cells, some of which eventually disintegrate. There is often a great deal of variation in the size and the structure

of gonads even in the same individual. The transformation of one of the two gonads is often more complete than that of the other. This is especially noticeable in the male type of intersexes. A number of cases were observed where in the same male intersex one gonad was testicular in character, while the other was an ovotestis.

In concluding this discussion of the development of gonads in *D. virilis* intersexes it may be pointed out that gonad development in *D. virilis* intersexes also differ from the intersexes of Lymantria and *D. melanogaster*. As has been shown by GOLDSCHMIDT and KERKIS (for *D. melanogaster*), gonads of the intersexes of these species either develop into those of the initial sex or into the gonads of an opposite sex, or are transformed into an organ which has the external and internal characteristics of both ovaries and testes.

IV. ABORTIVE GAMETOGENESIS IN INTERSEXES

The small cells found in the most distal part of the gonads of the intersexes during the larval stage are distinctly oogonial in various stages of development. In their nuclei about six heavily stained, small bodies can be seen, which probably represent five rod-shaped chromosomes and the nucleolus (Plate V, 1). The prochromosomes then become less distinct passing through the diffusion stage, while the nucleolus becomes a compact heavily staining body (Plate V, 2). After the oogonia undergo four mitotic divisions in succession, a group of sixteen young oocytes is formed from each. Several mitotic figures were observed in gonads of intersexes of all morphological types, which showed five pairs of rod-like and one pair of dot-like chromosomes (Plate V, 3). This comprises a typical female chromosome compliment.

The young oocyte nucleus is smaller, and its prochromosomes are more compact than in the nucleus of the oogonium (Plate V, 4). As in the young oogonia, the prochromosomes of the young oocytes pass through the diffusion stage, while the nucleolus becomes spherical and tends to move away from the nuclear membrane (Plate V, 5). After the diffusion stage the chromosomes of the oocytes appear as fine chromatic threads, corresponding to the leptene stage (Plate V, 6). After this stage the nucleus grows quickly and the chromatic threads undergo a marked shortening and twisting, thus realizing the pachytene and, perhaps, the strepsitene stages (Plate V, 7). Soon after the nucleolus moves toward the nuclear membrane, while the chromatin aggregates and forms a small closely bound network, which has a tendency to lie close to the nucleolus (Plate V, 8). All these stages of the growth of the oocytes are very similar to those found in the oocytes of normal females (GUYENOT and NAVILLE 1933). However, the oocytes of the normal female are very small, and only two

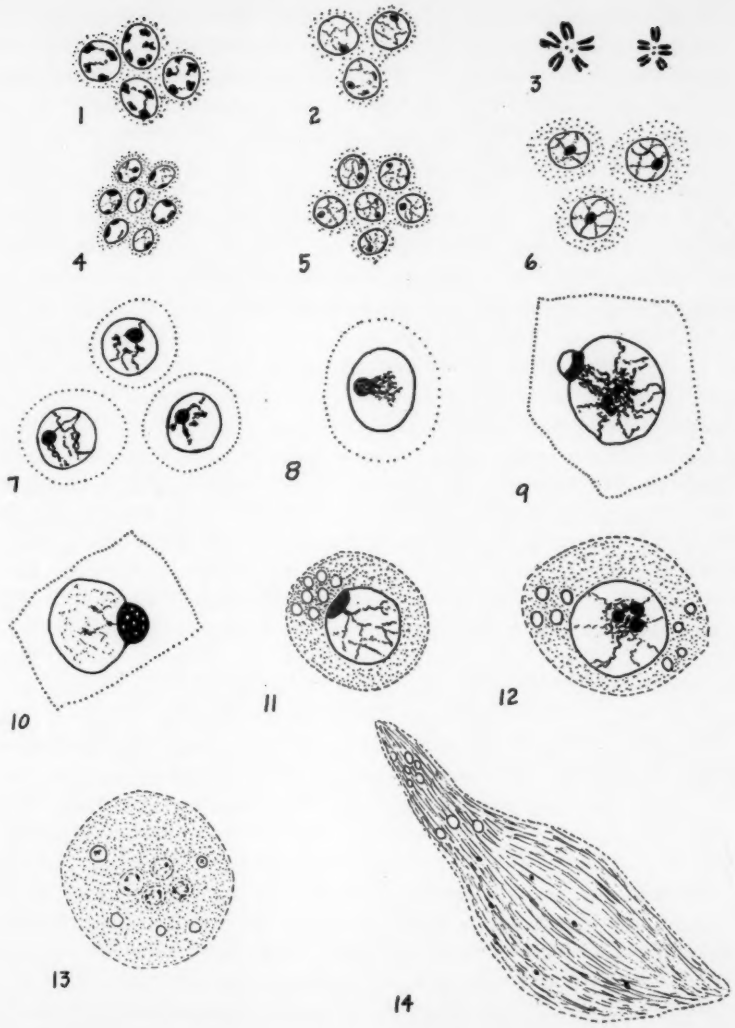

PLATE V.—Abortive gametogenesis in intersexes. Simplified camera lucida drawings. Magnification about 700 diameters. 1–2, young oogonia; 3, oogonial chromosomes at metaphase; 4, young oocytes; 5–8, "premeiotic" stages of the oocytes during which they gradually assume spermatocyte-like aspect; 9–10, oocytes transformed into spermatocyte-like cells; 11–14, disintegration of the transformed spermatocytes.

in the cysts undergo growth stages, while the oocytes of the intersexes are of considerable size, and all 16 in the cysts pass through these stages.

In the oocytes of normal females at this stage of growth, both the nucleolus and the chromatin content undergo the diffusion stage which lasts until the egg is about ready to be laid. The behavior of the oocytes in the intersexual gonads is quite different and resembles that found in the normal spermatocytes (METZ 1926). Instead of undergoing diffusion, the nucleolus continues to grow and moves rapidly toward the nuclear membrane, and forms a deep pocket in it, while the chromatin tends to resolve itself into a thread-like form (Plate V, 9). By this time the cells further increase in size and closely resemble spermatocytes of normal males. The diffusion stage follows soon and the chromatin appears very lightly stainable (Plate V, 10). The stages leading to the meiotic divisions can be seen in some of these spermatocyte-like cells. Some of them, and particularly their nuclei, become oblong. However, instead of the meiotic divisions these cells undergo a process of disintegration. The nucleolus appears to sink into the cytoplasm, and then is lost to view. This is followed by the elongation and flattening of these cells. A number of vacuole-like masses then appear in the cytoplasm (Plate V, 11–14). Often the process of disintegration is different. The nucleolus, instead of passing through the diffusion stage, moves away from the nuclear membrane. The pseudo-chromatic material around the nucleolus opens up and forms heavy, ribbon-like, deeply staining bodies. Pycnosis often is observed in this type of cells.

V. GENETICAL RELATIONSHIP BETWEEN THE VARIOUS MORPHOLOGICAL TYPES OF INTERSEXES

In the beginning of this work, an attempt was made to isolate lines producing predominantly one type of intersex. After but a few generations of selection lines 1, 3, and 4 were isolated. Since that time these lines have been kept in stock cultures. At various times attempts were made to improve these stocks, selecting only those giving more uniform intersexual types. However, it was noticed that this selection did not bring much improvement in the uniformity of the respective types. The following table shows the degree of variability in each of the isolated lines.

As can be seen from table 6, line 1 is the most variable of all the three lines, segregating approximately in a 1:1 ratio of type 1 and type 2. It must be remembered that there is no clear cut morphological difference between these two types, and therefore, the classification was not always accurate. Line 3 is the easiest to classify, and is comparatively stable. Only 19.6 percent of the intersexual population belonged to the other types. Line 4 is the most uniform, giving less than ten percent of the other types. However, the differentiation of type 4 from type 3 is not always

easy. This is mainly due to the variability in the degree of the fusion of the abdominal segments, and the gonad development. The intermediate number of abdominal segments between that of a true female and that of a true male can be found in both types. The degree of gonad development also often overlaps in both types.

TABLE 6

Variations in the morphological types of intersexes in the lines 1, 3, and 4.

LINES	NUMBER OF INTERSEXES DISSECTED	MORPHOLOGICAL TYPES 1	2	3	4
		%	%	%	
Line 1	192	44.7	53.7	1.6	—
Line 3	189	1.5	18.1	80.4	—
Line 4	184	—	1.3	8.3	90.4

The study of genetical relationship between the different morphological types, lines 1, 3 and 4 were intercrossed in various ways and also crossed to various stocks. The F_1, F_2 and the test cross generations from these crosses were raised and the intersexual population was studied. Very often the intersexes were dissected and the findings were recorded.

Quantitative inheritance

The study of the morphology of intersexes indicates that the various types of intersexes differ in the degree of development of the female and the male characteristics in them, the variations between such morphological types of intersexes being continuous. In other words, we are dealing with quantitative characteristics. It is well known that the study of the inheritance of such characteristics is extremely difficult due to many contributing factors. There usually are a larger number of genes involved, which contribute toward the expression and the degree of development of such characteristics (intersexuality in our case). On account of the lack of dominance of these genes, and their interaction, the division of the population into separate groups was often too artificial and did not represent the true genetical situation. If we add to this the consideration that sex is expressed in a number of complex organs, it is evident that the genetical analysis of intersexuality is very complicated. For these reasons this analysis is simplified by the separation of the intersexual population into four classes only. It is evident that by a classification restricted to a limited number of classes the error of sampling is increased, and the possibilities of detecting genes are lessened. However, by this method the complexity of genetical analysis due to incomplete dominance and gene interaction is also lessened. The purpose of this study, therefore, is rather to reveal the

minimum number and for that reason probably the most important genetical factors affecting the degree of intersexuality, than to find the possible maximum number of such factors, which is obviously beyond the scope of the present work.

The analysis of the data pertaining to the genetical relationship between the various morphological types of intersexes will be treated in three separate groups, due to the different types of crosses and somewhat different results obtained in each group.

A. Crosses between lines 4, 3 and 1

Cross between lines 4 and 3. In the F_1 from the cross between lines 4 and 3, 129 intersexes were obtained. Of this number 110 were classified as being intermediate between types 3 and 4 (designated as type $3\frac{1}{2}$), seventeen intersexes belonged to type 4, and two to type 2 (table 7a). The type $3\frac{1}{2}$

TABLE 7

Summary of crosses between lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES	MORPHOLOGICAL TYPE				
					1	2	3	$3\frac{1}{2}$	4
a 3×4 F_1	10	336	317	129		2		110	17
b 3×4 F_2	3	184	108	69			17	40	12
c $3\times(3\times4)$	7	338	188	159*		5	19	27	
d $(3\times4)\times4$	4	194	160	73				40	33
e 1×3 F_1	8	287	179	101*		77	12		
f 1×3 F_2	24	1231	893	343*	43	186	59		16
g $(1\times3)\times1$	10	586	344	187*	57	84	14		
h $(1\times3)\times3$	11	565	294	246	3	119	120		4
i 1×4 F_1	11	365	291	146		135	11		
j 1×4 F_2	8	299	242	202	23	97	36		46
k $(1\times4)\times1$	2	87	52	29	9	17	5		
l $(1\times4)\times4$	4	223	144	82		33	13		36

* Only part of the intersexual population from these crosses were recorded as to the morphological types.

intersexes were noticeably different from either type 3 or 4. The genitalia in the type $3\frac{1}{2}$ intersexes were more completely twisted than in type 3, but not reaching an almost symmetrical position as in type 4. Also the abdominal segments of type $3\frac{1}{2}$ were less completely fused than in type 4. However, the classification of the intermediate type $3\frac{1}{2}$ was attempted only in crosses where only the types 3 and 4 were involved. It was difficult to identify this intermediate type among the intersexual population from the crosses where type 1 was involved, because of the variability of types 3

and 4 themselves, and also because other genetical factors are involved in such crosses.

In the F_2 from the cross of lines 3×4, 69 intersexes were obtained, of which 17 belonged to type 3, 40 to type $3\frac{1}{2}$, and 12 to type 4 (table 7b). The segregation of the intersexual population in the F_2 of this cross approximates that of 1:2:1 ratio, indicating that line 4 differs genetically from line 3 either by a single semi-dominant modifier of the *ix*^{m} gene, or by a different allele of *ix*^{m}. The segregation of the intersexual population in the test-cross is in agreement with either assumption. From the cross of line 3 females to F_1 (3×4) males, 159 intersexes were obtained, of which 51 were taken at random and dissected (table 7c). Of these 51 intersexes, five were classified as type 2, 19 type 3, and 27 type $3\frac{1}{2}$. Considering the five type 2 intersexes as variations of type 3, it can be assumed that 1:1 segregation of types 3 and $3\frac{1}{2}$ took place. Similarly from the test-cross of F_1 (3×4) females to males of type 4, 73 intersexes were obtained, 40 of which were of type $3\frac{1}{2}$ and 33 of type 4 (table 7d). Again a 1:1 segregation occurred, but this time of types $3\frac{1}{2}$ and 4.

Cross between lines 3 and 1. The segregation of the intersexual population in the crosses where line 1 was involved was very conflicting. Therefore no attempt will be made to interpret the genetical difference (if such exists) between types 1 and 2 and these two types will be considered together.

From the cross between lines 1 and 3, 101 intersexes were obtained in the F_1 (table 7e).[5] Out of the 89 intersexes which were dissected and recorded, 77 were classified as belonging to type 2 and 12 to type 3. Considering the variations in the morphology of intersexes of the parental lines 1 and 3 (see table 6), the F_1 intersexual population from this cross can be considered as being intermediate between that of the parental lines. In other words, the dominance of one type of intersex over the other is lacking in the cross between lines 1 and 3, as is also true in the case of the cross between lines 3 and 4.

From the 24 F_2 cultures from this cross 343 intersexes were obtained (table 7f). Of these, only 304 were classified and 229 of them were either types 1 or 2, 59 of type 3, and 16 of type 4. The intersexual population from this cross segregated approximately in 12:3:1 ratio of the types 1 and 2 (taken together) and 3 and 4 respectively. This result is of considerable interest. The type 4 intersex is a new type in this cross, resembling neither of the parental types. Its appearance in 1/16 in the F_2 generation indicates that the various morphological types of intersexes are not due to various alleles of the *ix*^{m} gene, but are the result of *ix*^{m} modifiers. There

[5] In the F_1 and test-crosses in these series of experiments the ratio of males, females and intersexes departs from both the 2:1:1 and the 4:3:1 ratios due to the fact that no attempt was made to group separately the cultures giving either of these two ratios.

are probably two modifying factors involved, type 4 being a double recessive (*ab*), and types 1 and 2 being either double dominant or dominant for at least one modifier (*AB* or *Ab*), and type 3 dominant for the other modifier (*aB*).

Since the test cross was made only to parental types 1 and 3, the hypothesis was tested only partially. From the cross of F_1 females to males from line 1, 187 intersexes were obtained most of which belonged either to types 1 or 2 (table 7g). While from the cross of F_1 males to females of line 3, 246 intersexes were obtained. Of these, three belonged to type 1, 119 to type 2, 120 to type 3 and four to type 4 (table 7h). This is an almost perfect 1:1 segregation of types 2 and 3. This test supports the assumption of one gene difference between lines 1 and 3.

Cross between lines 1 and 4. The result of crosses between lines 1 and 4 substantiates the assumption of two *ix*^{m} modifiers. However, further modification of the dihybrid ratio was found in this type of cross. In the F_1 from the cross between lines 1 and 4, 146 intersexes were obtained. Of this number 135 were of type 2, and 11 of type 3 (table 7i). This result is similar to that obtained in the F_1 from the cross between lines 1 and 3. This indicates that dominance of one morphological type over the other is lacking in the cross between lines 1 and 4, as was true also in the case of crosses between lines 1×3 and 3×4.

The F_2 intersexual population from this cross segregated into 120 intersexes of types 1 and 2, 36 of type 3, and 46 of type 4 (table 7j). This segregation approximates a 9:3:4 ratio. The appearance of type 4 intersex in the 4/16 proportion among the intersexual population from this cross indicates that the modifier *A* alone has no effect on the *ix*^{m} gene.

The result of the test cross, and especially the cross to the parental line 4, substantiates this hypothesis. The cross of F_1 females to the males of line 4 resulted in 82 intersexes (table 7l). Of these 33 belonged to type 2, 13 to type 3, and 36 to type 4. Considering 13 intersexes of the type 3 as being variations of the type 4, a very good 1:1 segregation of types 2 and 4 occurred in this case. From the cross of the F_1 females to the males of the parental line 1 only 29 intersexes were obtained most of which belonged either to type 1 or 2, which is in agreement with expectation (table 7k).

Summing up the results of the crosses between lines producing various morphological types of intersexes it may be assumed that intersexes of types 1 and 2 are of *AB* constitution, of type 3 *aB* ,and type 4 of *Ab* and *ab*.

However, the hypothesis does not meet the crucial test. If line 3 intersexes are of *aB* constitution, and line 4 intersexes are *Ab* or *ab*, then from the majority of the F_1 crosses between lines 3 and 4, the intersexes should be of types 1 and 2 (*AB*). However, from several of these crosses the intersexes were of type $3\frac{1}{2}$, as if line 4 intersexes were of *ab* constitution. At the

present time the author is not in a position to offer another explanation for the segregation of the morphological types of intersexes. The assumption of the presence of two modifying factors will be retained as a working hypothesis until additional experimental data are available. The results of the crosses of lines 1, 3 and 4 with rounded and puffed stocks, which will be discussed later, support this assumption. On the other hand, at the present there is no adequate explanation for the appearance of intersexes of type $3\frac{1}{2}$ in the F_1 from the cross between lines 3 and 4. There is one possibility, however, not very probable, which may explain this inconsistency, namely, that *aaBB* and *aaBb* males of line 4 are sterile, so that the line 4 males used in the cross were of *aabb* constitution. Then from the cross between *aabb*×*aaBB* flies the F_1 intersexual population genotypically is *aaBb* and phenotypically should be intermediate between intersexes of types 3 and 4.

B. Crosses between lines 4, 3 and 1 and rounded and puffed stocks

The conflicting data as to the segregation of the different morphological types of intersexes in the crosses described above suggested further tests with different stocks not carrying the ix^m gene. Rounded and puffed, which were used for this purpose, are both dominant genes of the second chromosome. Since the result of the crosses between intersex producing lines and rounded (*R*) and puffed (*Pu*) stocks is similar, the data will be considered together.

From the cross between line 4 and *R* and *Pu* stocks, 322 intersexes were obtained in the F_2 (table 8a, b, and c). Of these 301 were classified as type

TABLE 8

Summary of the F_2 crosses between R and Pu stocks and lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES	MORPHOLOGICAL TYPE 1	2	3	4
a *R* ♀×line 4 ♂	24	821	691	195		3	10	182
b *Pu* ♀×line 4 ♂	12	504	425	127			8	119
c	37	1325	1116	322		3	18	301
d *R* ♀×line 3 ♂	11	419	333	102		6	64	32
e *Pu* ♀×line 3 ♂	29	1378	1048	335		25	174	82
f	40	1797	1381	437		31	238	114
g *R* ♀×line 1 ♂	25	1157	960	313	29	149	74	61
h *Pu* ♀×line 1 ♂	21	844	600	145	12	69	35	29
i	46	2001	1560	458	41	218	108	90

4, 18 as type 3, and three as type 2. Since the variability of intersexes in the F_2 of this cross does not exceed that of the line 4 itself, it can be assumed that the F_2 intersexual population morphologically is the same as the parental line 4.

From the cross between line 3 and rounded and puffed stocks 437 intersexes were obtained in F_2. Of these 31 were classified as belonging to type 2, 238 to type 3, and 114 to type 4 (table 8d, e, and f). Assuming that 31 intersexes of type 2 are variations of the type 3 and grouping them together, there will be 269 intersexes of type 3 and 114 of type 4, which approximates a 3:1 ratio.

The F_2 from the cross between line 1 with *R* and *Pu* stocks gave 458 intersexes (table 8g, h, and i). Of these 41 belonged to type 1, 218 to type 2, 108 to type 3, and 90 to type 4. Considering types 1 and 2 altogether, the intersexes segregated into 259:108:90, which significantly deviated from the expected 9:3:4 ratio. However, since this segregation closely approximates a 9:7 ratio the deviation of the data from a 9:3:4 ratio might be due to faults in the classification of lines 3 and 4.

The result from these crosses seemed to be in agreement with that obtained from intercrosses between lines 1, 3 and 4. The most important point brought out by these crosses is the nature of the ix^m gene itself. At least one modifier is required to produce an intersex of other than the extreme male type (type 4). In other words, when the ix^m gene is not influenced by its specific modifying factors, females homozygous (ix^m/ix^m) become converted into sterile males. Therefore the ix^m gene is the gene for maleness. The masculinizing effect of this gene is so strong that it overrides the 2X:2A mechanism, the balance of which is on the female side.

C. Dominant suppressors of ix^m

In connection with linkage studies, in which the genes *sv*, ix^m, and *G* were involved, it was observed that the intersexual population in those crosses was deficient. The result of these and other similar crosses will now be discussed in detail.

The F_1 females, of $++G/sv\ ix^m+$ genotype, were crossed to *sv* males from the stock segregating for intersexes. It was expected that from these crosses cultures throwing intersexes would segregate either in a 1:1 or a 3:1 ratio of normal females and intersexes. However, out of 56 cultures from this cross, only in two, the ratio of females to intersexes approximated that of 3:1 (table 4). In the rest of the cultures the female to intersex ratio is modified into 7:1, 15:1 and even 31:1. This can be explained on the assumption that the *G* stock used in the cross carries perhaps two or more dominant suppressors of the ix^m gene, each of which alone is capable of nullifying the effect of the ix^m gene. As our observations suggest, the

ix^m/ix^m females homo- or heterozygous for these suppressors become normal females. However, no attempt was made to study the internal organs of these females, nor to test their fertility.

In order to find the linkage relation of these suppressors to the ix^m or to each other, a reciprocal cross was made. The *G* females were crossed to males from lines 1 and 3. Then F_1 males from these crosses were outcrossed to *v mt* females of line 4. Line 4 females were used in preference to the females of lines 1 and 3 for the following reasons. While records were made for the three point test, it was observed that most of the intersexual population from this cross consisted of an extreme male type. Therefore it was considered desirable to use *v mt* females of line 4 for the test cross so that the males and intersexes could be easily distinguished. From these crosses 609 *v mt* males, 603 females, and 86 wild type intersexes were obtained (table 9). Both males and females from these crosses also segregated for

TABLE 9

Summary of the crosses between v mt line 4 females and F_1 males from the cross G females with males of lines 3 and 1.

F_1 MALES FROM THE CROSS	NUMBER OF CULTURES	*v mt* AND *v mt G* MALES	+ AND *G* FEMALES	+ INTERSEXES	MORPHOLOGICAL TYPES 1	2	3	4
G ♀ × line 3 ♂	9	261	272	38			16	22
G ♀ × line 1 ♂	11	348	331	48		5	7	36
	20	609	603	86				

G, but no record was kept of this. However all intersexes were examined for eye color and were found to be of the wild type. Though the number of cultures is small, the results indicate that the female and intersexual population from these crosses segregate mostly into 7:1, and perhaps 15:1 ratios. This would indicate that some of the suppressors present in the *G* stock might be linked with the third chromosome, or linked together.

Further tests were made between intersex-producing lines and non-related stocks in order to reveal the presence of the ix^m suppressors. It was found that in a number of crosses the ratio between female and intersexual populations was altered from that of 3:1 and 1:1. Only two other crosses, with American wild stock and mt^4, the data of which are more complete, will be considered here.

The American wild type females were crossed to males from intersex producing lines 4, 3 and 1. Then F_1 males from these crosses were crossed to *v mt* females of line 4. From these crosses altogether 57 cultures were obtained, which gave 1735 *v mt* males, 1675 wild type females and 211 wild type intersexes (table 10). The ratio of males, females and intersexes from

TABLE 10

Summary of the test crosses of F_1 males (from the crosses between American wild type females and the males from intersexual lines 4, 3, and 1) to v mt line 4 females.

F_1 MALES FROM CROSSES	NUMBER OF CULTURES	*v mt* MALES	WILD TYPE FEMALES	WILD TYPE INTER-SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (+ ♀ × line 4 ♂)	7	232	202	27			2	25
b (+ ♀ × line 3 ♂)	27	815	796	106		5	44	57
c (+ ♀ × line 1 ♂)	23	688	677	78	8	8	21	78
	57	1735	1675	211				

these crosses approximates that of an 8:7:1 ratio, indicating that the wild stock is homozygous for at least one suppressor. From the F_2 cross altogether 45 cultures were obtained which gave 1738 males, 1814 females and 77 intersexes (table 11). The ratio of females to intersexes in these crosses

TABLE 11

Summary of the F_2 crosses between American wild type females and the males of intersexual lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER-SEXES	MORPHOLOGICAL TYPE			
					1	2	3	4
a (+ ♀ × line 4 ♂)	8	250	268	14			3	11
b (+ ♀ × line 3 ♂)	17	619	684	23		2	16	5
c (+ ♀ × line 1 ♂)	20	869	862	40	4	9	11	16
	45	1738	1814	77				

on the average is 25:1, though in individual cultures it appears to be modified still further. The result of these crosses indicates that the wild stock probably carried more than one suppressor of *ix*^{m}. The exact number cannot be estimated from the data.

The data in regard to the *mt*^{4} stock are less complex and indicate the presence of only a single *ix*^{m} suppressor in this stock. The *mt*^{4} females were crossed to males from lines 4, 3 and 1. The F_1 males or females from these crosses were back crossed to line 4 stock. From these crosses altogether 64 cultures were obtained, which gave 2678 males, 2519 females and 319 intersexes (tables 12 and 13). The ratio of females to intersexes approximates that of 7:1. The result of F_2 crosses substantiates the assumption of the presence of only one *ix*^{m} suppressor in the *mt*^{4} stock. The 78 F_2 cultures from these crosses gave 3600 males, 3972 females, and 245 intersexes (table 14). The ratio of females and intersexes approaches closely that of 15:1

TABLE 12

Summary of test crosses of F_1 males (from the cross between mt^4 females and the males from intersexual lines 4, 3, and 1) to v mt line 4 females.

F_1 MALES FROM CROSSES	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES	MORPHOLOGICAL TYPE 1	2	3	4
a (mt^4 ♀ ×line 4 ♂)	4	113	101	10				10
b (mt^4 ♀ ×line 3 ♂)	19	505	526	78		6	40	32
c (mt^4 ♀ ×line 1 ♂)	19	553	558	50	2	5	8	35
	42	1171	1185	138				
From Table 13	22	1501	1334	181				
	64	2678	2519	319				

TABLE 13

Summary of test crosses of F_1 females (from the cross between mt^4 females and males from intersexual lines 4, 3, and 1) to v mt line 4 males.

F_1 FEMALES FROM CROSSES	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES	MORPHOLOGICAL TYPE 1	2	3	4
a (mt^4 ♀ ×line 4 ♂)	12	941	786	98			13	85
b (mt^4 ♀ ×line 3 ♂)	4	168	157	21		1	8	12
c (mt^4 ♀ ×line 1 ♂)	6	392	391	62	1		13	48
	22	1501	1334	181				

TABLE 14

Summary of the F_2 crosses between mt^4 females and males of the intersexual lines 4, 3, and 1.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES	MORPHOLOGICAL TYPE 1	2	3	4
a (mt^4 ♀ ×line 4 ♂)	29	1481	1576	91	1		11	79
b (mt^4 ♀ ×line 3 ♂)	15	515	634	43			10	33
c (mt^4 ♀ ×line 1 ♂)	34	1604	1762	113	5	6	27	75
	78	3600	3972	245				

Morphology of intersexes in crosses with stocks homozygous for suppressors

In the discussion of results of crosses in which suppressors were involved, the morphology of intersexes has not been considered. This merits special consideration. In table 15 the results of crosses between lines 4, 3 and 1 with wild and mt^4 stocks are summarized. The F_2 intersexual population

from the cross between line 4 and wild and mt^4 stocks is mostly of the extreme male type 4 (table 15a and b). This is in agreement with the results of crosses between line 4 and the other stocks free of ix^m suppressors, as it has been described above. The F_2 intersexes from the cross between

TABLE 15

Summary of the F_2 cross between lines 4, 3, and 1 and wild and mt^4 stocks.

TYPE OF CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTERSEXES	MORPHOLOGICAL TYPE 1	2	3	4
Line 4								
a ×wild	8	250	268	14			3	11
b ×mt^4	29	1481	1576	91	1		11	79
					1		14	90
Line 3								
c ×wild	17	619	684	23		2	16	5
d ×mt^4	15	515	634	43			10	33
						2	26	38
Line 1								
e ×wild	20	869	862	40	4	9	11	16
f ×mt^4	34	1604	1762	113	5	6	27	75
					9	15	38	91

line 3 and wild and mt^4 stocks consisted of two intersexes of type 2, 26 of type 3, and 38 of type 4 (table 15c and d). The predominance of type 4 intersexes over those of type 3 can be easily accounted for, as it has been shown, there is probably one gene difference between lines 3 and 4; dominance of one type over the other is lacking. The intersexes heterozygous for this modifier are morphologically intermediate between types 3 and 4. In some crosses these intersexes morphologically are closer to type 3 and are classified with them. In these crosses some of the intermediate types morphologically are closer to type 4 and are classified together with this type. But the F_2 intersexual population from the crosses between line 1 and wild and mt^4 stocks is quite different from that when line 1 was crossed to stocks free from suppressors. The F_2 intersexual population from the cross between line 1 and *R* and *Pu* stocks segregated into a 9:3:4 ratio of types 1 and 2 (taken collectively), 3 and 4, respectively. Whereas in the F_2 from the crosses of line 1 with wild and mt^4 stocks the situation is quite different. Out of 153 intersexes from these crosses only nine belonged to type 1, 15 to type 2, and the majority of the intersexes were of types 3 and 4 (38 of type 3 and 91 of type 4), (table 15e and f). The segre-

gation of F_2 intersexes from these crosses is almost in reverse order from that found in the F_2 from the cross between line 1 and *R* and *Pu* stocks.

The segregation of the intersexual population in the test cross from the matings in which suppressors were involved correspond to the result obtained in the F_2 from these matings. As was stated before, F_1 males from the cross between lines 4, 3 and 1 with wild and mt^4 stocks were outcrossed to *v mt* females from the line 4. This was considered desirable in view of the fact that a majority of the intersexes from such crosses were of the extreme male type. The introduction of the sex-linked genes permitted unmistakable separation of normal males from male-like intersexes. The summary of the results of these and reciprocal crosses are given in table 16. The test

TABLE 16

Summary of the test crosses. The wild and mt^4 stocks were crosses to intersexual lines 4, 3, and 1. The F_1 flies from these crosses were outcrossed to line 4.

F_1 PARENT USED IN CROSS	NUMBER OF CULTURES	MALES	FEMALES	INTER-SEXES	MORPHOLOGICAL TYPE 1	2	3	4
a F_1 (wild×line 4)	7	232	202	27			2	25
b F_1 (mt^4×line 4)	16	1045	887	108			13	95
							15	120
c F_1 (wild×line 3)	27	815	796	106		5	44	57
d F_1 (mt^4×line 3)	23	673	683	99		7	48	44
						12	92	101
e F_1 (wild×line 1)	23	688	677	78		8	21	49
f F_1 (mt^4×line 1)	25	945	919	112	3	5	21	83
					3	13	42	132

cross population from the crosses in which lines 4 and 3 were involved segregated in a manner similar to that found from the crosses of these lines and stocks free from suppressors (table 16 a–d). The test cross intersexual population in which line 1 was involved again is exceptional, as it was in the case of the F_2 intersexes. Out of 190 intersexes obtained from the test-cross, only three belonged to type 1, 13 to type 2, 42 to type 3, and 132 to type 4 (table 16 e and f). Again type 4 predominates, which is contrary to expectation.

The predominance of the type 4 intersexes has also been observed in the progeny of crosses between intersexual lines and other stocks carrying ix^m suppressors. However, accurate records as to the morphology of the intersexual population from these crosses have not been taken. At the present the author has no adequate explanation for the predominance of

the male type of intersexes over those of the female-like and hermaphrodites in the progeny from these crosses. Further research undoubtedly would be desirable.

VI. DISCUSSION

The intersexes in *Drosophila virilis* have an entirely normal chromosomal constitution, and therefore may be called diploid intersexes. The intersexuality in this species is the result of the interaction of the mutant autosomal gene ix^m and its modifiers with the rest of the 2X:2A chromosomal complex. The females homozygous for ix^m and lacking modifiers become sterile males. The presence of one or more specific modifiers in the ix^m/ix^m females results in incomplete reversion and various degrees of hermaphroditism. The addition to the genotype of these individuals of one or more suppressors inhibits the action of the ix^m gene and its modifiers, resulting in the development of normal females. Also, 2X:2A individuals develop into normal females when they are heterozygous for ix^m ($ix^m/+$) irrespective of their constitution in regard to the ix^m modifiers. The 1X:2A individuals are not affected either by the ix^m gene or by its modifiers.

The nature of the ix^m gene and its normal allele, and their possible significance in the sex-determining mechanism has been discussed (LEBEDEFF 1938b) and need not be repeated here. It should be pointed out that these considerations are purely hypothetical. It is hoped that further research with these intersexes may contribute to our understanding of the mechanism of sex determination. However, even at the present stage of our knowledge of these intersexes it might be assumed that in the sex-determining mechanism of Drosophila the main male (*M*) sex gene is present. The indirect evidence from *D. melanogaster* suggests the presence of the main female (*F*) sex gene (PATTERSON, STONE and BEDICHEK 1937). The interaction or the balance of these genes in the sex determination is subjected to the effect of their specific modifiers, the number of which is probably large.

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SUMMARY

Diploid intersexuality in *Drosophila virilis* is described in detail.

The third chromosome gene (ix^m), whose locus on the chromosome is known, converts homozygous females (ix^m/ix^m) into sterile males. The gene has no effect on males, and the cross between $ix^m/+$ ♀ and ix^m/ix^m ♂ produces males, females and intersexes in the ratio of 2:1:1. The cross of $ix^m/+$ ♀ × $ix^m/+$ ♂ produces males, females and intersexes in the ratio of 4:3:1.

However, these ratios are often modified, when intersex producing stocks are outcrossed to unrelated stocks, by the presence in these stocks of one or more dominant suppressors of the ix^m gene.

Two or more modifiers delay the transformation of the ix^m/ix^m females into males, resulting in various degrees of intersexuality. The genetical evidence suggests that these modifiers are incompletely dominant.

Morphologically intersexes vary from female-like to nearly male-like. The specimens intermediate between these two extremes possess well developed female and male sexual characteristics, including external and internal sexual organs, and gonads.

Genetical and morphological studies suggest that intersexes of all morphological types start their development as females and then from a certain time, known as the turning-point, continue to develop in a male direction. The development of the male sexual system, however, does not interfere with that of the female, resulting in the development of hermaphrodites of various degrees.

The gonads in all morphological types of intersexes start their development as ovaries. In the female-like intersexes ovaries continue their development, though it is retarded in various degrees. In hermaphrodites after the occurrence of the "turning-point" ovaries are transformed into ovotestes. In the male-like type of intersex ovaries are gradually transformed into testis-like organs.

During the transformation of ovaries into ovotestes or into testis-like organs, the female germinal cells are gradually transformed into spermatocyte-like cells, some of which eventually disintegrate.

The intersexes of all morphological types are sterile since gametogenesis is abortive. Meiosis has not been observed in the gonads of intersexes.

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THE INHERITANCE OF TAILLESSNESS (ANURY) IN THE HOUSE MOUSE. II. TAILLESSNESS IN A SECOND BALANCED LETHAL LINE

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INTRODUCTION

IN THE house mouse several mutations affecting the development of the axial skeleton have occurred at or near one genetic locus, and combinations of these have been shown to lead to failure of the posterior parts (tail and sacral region) to complete their development normally. Such phenotypes are most readily classified according to modifications of the tail, and have been referred to as short-tailed (brachyuric) or tailless (anuric). Examination of embryos at various stages has shown that the parts that are missing in late embryos and newborn young are present in earlier stages, but after a given period in development cease their growth, are dedifferentiated and resorbed. In general the notochord in the early embryo is abnormal or absent and axial parts lacking a normal notochord are subject to such regression.

Genetic evidence showing that the brachyuric forms are heterozygous for a lethal mutant factor was provided by DOBROVOLSKAIA-ZAWADSKAIA, KOBOZIEFF, and VERETENNIKOFF (1934), and an embryological study of such hetero- and homozygotes has been made by CHESLEY (1935). A description of the tailless lines and a preliminary study of the inheritance of this character have been reported by KOBOZIEFF (1935); and an embryological study by GLUECKSOHN-SCHOENHEIMER (1938). Proof that one tailless line (A) contains the balanced lethals *T*, homozygous lethal at about 11 days in utero, and t^0, homozygous lethal at about the time of implantation of the embryo, has been given by CHESLEY and DUNN (1936); while DUNN (1937) has reported preliminary data showing that a similar interpretation applies to a second tailless line in which, however, the recessive lethal (t^1) is different from that found in line A. The homozygote t^1t^1 has been shown by GLUECKSOHN-SCHOENHEIMER (1938) to die before implantation.

Thus three mutations have been described each of which when homozygous has an early lethal effect. These mutations have shown no crossing over with each other, and this, together with the fact that each appears to affect the early development of the notochord and axial structures in

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a similar way, has led to the assumption that the three constitute a series of multiple alleles. If this is so, then the relations of the alleles in respect to their effects on morphogenesis and on viability present an interesting problem, since compounds of different lethal alleles are viable (Tt^0, Tt^1, t^1t^0). In addition, the t^0 and t^1 mutations apparently segregate normally from female heterozygotes and compounds ($+t^0$, $+t^1$, Tt^0, Tt^1, t^0t^1) while males of genotypes $+t^0$ and Tt^0 appear to transmit t^0 in much higher frequency than its + or T allele (CHESLEY and DUNN 1936). The last-named authors have suggested that this behavior is due to an effect of t^0 on spermatogenesis, and this is made more likely by the discovery that t^0t^1 males are entirely sterile, although t^0t^1 females are fertile (DUNN 1937).

The opportunity is seldom given to compare in the same material the effects of mutations at or near the same locus on developmental processes concerned with several fundamental aspects of the organism, such as morphogenesis, viability and gametogenesis. An intensive study of this case has therefore been undertaken. Sufficient data on the balanced lethal condition in the "A" tailless line (Tt^0) have been given for present purposes; in this paper, breeding data on the second balanced lethal line, "29" (Tt^1), are given and the inheritance of the t^1 mutation of this line is discussed.

METHODS

The following precautions were observed in breeding and in recording observations. Several individually marked females were usually bred in one pen to one male. Pregnant females were isolated several days before the birth of the litter and all litters were born in such isolation pens. Classification of tail characters was always made shortly after birth. Parturition usually occurs at night or in the early morning and litters are classified at about 9 A.M. There is thus little opportunity for mistake in pedigree, or for differential depletion of the litter by destruction on the part of the mother. Our experience with these methods indicates that practically all animals born can be found and accurately classified. Records of partially destroyed animals are not included in the summaries given here.

GENETIC ANALYSIS OF LINE 29 TAILLESS

It has been shown (KOBOZIEFF 1935, DUNN 1937) that *inter-se* matings of tailless animals of Line 29 produce only tailless progeny, rarely with a small bony stump in the position of the tail. Outcrosses of such tailless animals to other stocks (normal, Brachy, tailless of Line A) show that they are always heterozygous and breed true because both mutations involved are lethal when homozygous. The balanced lethal condition maintains itself because no crossing over occurs between the two mutations; it is

assumed that they fail to cross over because they are alleles. These hypotheses were derived from preliminary data (DUNN 1937) and have now been tested by additional experiments.

Balanced lethal condition. The tailless Line 29 has bred true for five years during which time 96 litters have been recorded containing 357 young at birth, all of which were tailless (table 1, exp. 1). Of these 11 had small non-flexible bony stumps. The latter did not differ in breeding behavior from other tailless animals of this line.

The matings of tailless by tailless produce small litters, but conclusive evidence as to the segregation of two lethals in such matings cannot be derived merely from data on litter size. The tailless animals of this line are in general smaller and less vigorous than other normal stocks. Their genetic constitution, apart from the two mutations affecting the tail, was probably different from that of other normal stocks, at least in the early generations. During the last three years, the stock has been repeatedly backcrossed to a normal inbred (Bagg albino) stock, and the tailless genotype recovered; so that now, after five such backcrosses, the genetic constitution of this line is more nearly comparable with the Bagg albino and Brachyury lines. The litter size of Line 29 is still much smaller (average about four) than in the normal Bagg albino line (average about eight).

More direct confirmation of the balanced lethal hypothesis is obtained from dissection of tailless females fertilized by tailless males. In the uteri of such females after the eighth day there are found two classes of implanted embryos: homozygotes (TT) which die at the end of the tenth day, and viable embryos which become tailless (Tt^1). GLUECKSOHN-SCHOENHEIMER (1938) has shown that the t^1t^1 homozygotes die before implantation.

That T and t^1 do not cross over is shown by the absence of crossover zygotes in *inter-se* matings of tailless. Crossing over would produce gametes Tt^1 and $++$. The latter, in union with $++$ or t^1 gametes would produce zygotes with normal tails; in union with T gametes they would produce Brachy zygotes. Such have not been found.

Results of crossing Line 29 tailless by normal

The cross of tailless by normal was made reciprocally and in each case two classes of offspring, Brachy and normal, were produced, showing that the tailless parents were heterozygous for a dominant mutation for short tail. Tests of the Brachy offspring by *inter-se* matings and by Brachy stock showed them to be $T+$. The normals, as shown below, were $+t^1$. The proportions of these two classes of offspring were different in the reciprocal crosses. From matings of tailless females by normal males, normal and Brachy offspring appeared in equal numbers, the expected result (table 1, exp. 2). The cross of normal females by tailless males gave 647

normal, 85 Brachy and 3 tailless (exp. 3). Two of the latter class were tested and proved to be $T+$, that is they were Brachys with extreme tail reduction, thus overlapping the tailless phenotype. The ratio of Brachy to normal from tailless fathers was thus about 1:7.5, instead of 1:1.

TABLE 1

Results of matings of Line 29 tailless mice inter-se and with normals and Brachys.

EXP. MATING	NORMAL ♀	NORMAL ♂	NORMAL ?	NORMAL TOTAL	BRACHY ♀	BRACHY ♂	BRACHY ?	BRACHY TOTAL	TAILLESS ♀	TAILLESS ♂	TAILLESS ?	TAILLESS TOTAL	TOTAL
1. Tailless×tailless													
(a) Inbred stock									95	102	12	209	209
(b) Segregates inter-se									77	68	3	148	148
2. Tailless ♀×normal ♂	41	24		65	32	25	3	60					125
3. Normal† ♀×tailless ♂	328	283	36	647	44	39		85	3*			3	735
4. Tailless ♀×Brachy ♂	14	6		20	9	4		13	6	11		17	50
5. Brachy ♀×tailless ♂	111	107	13	231	23	25		48	112	98	10	220	499
6. F_1 normal ♀×Brachy ♂	361	383	20	764	201	177	7	385	221	181	9	411	1560
7. Other normal ($+t^1$) ♀ ×Brachy ♂	104	92		196	68	69		137	78	49		127	460
8. Brachy ♀×$+t^1$ normal ♂	913	896	11	1820	168	208		378	689	691		1386	3584

* Genetically 2 of these were $T+$, that is, Brachys with extreme reduction of the tail; the other one was sterile.
† Inbred Bagg albino females; by other normal females, three tailless males gave 45 n, 9 Br.

Further analysis of the inheritance of T and t^1 was made by studying the normal progeny of the cross of tailless male by normal female. It had to be determined first whether this extremely aberrant ratio was a genotypic or merely a phenotypic one made abnormal by the failure of the deficient class (Brachy) to manifest its genetic constitution. This last possibility was not a likely one since it implies errors in classification (overlapping variability) as between Brachy and normal. The normal ratios obtained from tailless females (sibs of the tailless males used in the reciprocal cross) indicate that there was little or no classification error or overlapping variability. Nevertheless two experiments should provide a decisive answer in this case and should in addition yield some evidence on whether crossing over occurs between T and t^1.

1. If the ratio is a genotypic one, then all of the F_1 normal-tailed offspring should be $+t^1$. One hundred thirty four normal-tailed offspring (109 ♀♀, 25 ♂♂) from crosses of tailless males by normal females were tested by crossing with Brachy. Each of them produced normal, Brachy and *tailless* progeny, proving that all of these F_1's were $+t^1$. In addition, six F_1 normals (four ♀♀, two ♂♂) from the cross of tailless male by Brachy female similarly proved to be $+t^1$. Thus 140 F_1 normals were shown to be $+t^1$ without exception. The F_1 ratio is therefore a genotypic one and represents the actual frequencies of the genotypes $+t^1$ and $+T$ among the progeny of tailless males.

2. As an additional test other normal-tailed F_1's were crossed with normals. If any F_1 normal was genotypically $+T$ but failed to manifest the

Brachy phenotype such test crosses should produce some Brachy offspring. Fourteen F_1's so tested produced only normal offspring. The test requirement was a minimum of 11 offspring per F_1 individual.

These tests showed also that 140 gametes from tailless males carried t^1, while 87 gametes (85 F_1 Brachys and two tailless which proved to be $T+$) carried T, and only these two types of gametes were found. This means that no crossing over occurred between T and t^1 in 227 tested cases. The 357 tailless offspring from matings of tailless by tailless also constitute negative tests for crossing over.

Differences in viability as between normal and Brachy offspring cannot explain the abnormal ratio since (1) the same two classes segregated from the reciprocal cross (tailless female by normal male) in a normal ratio; (2) the litter sizes obtained from the cross normal female by tailless male were normal and high (average 7.5 per litter).

Segregation of t^1 in later generations

The test of F_1 normal females by Brachy males ($+T$) showed normal segregation of t^1, producing about $\frac{1}{2}$ normal ($++$, $+t^1$), $\frac{1}{4}$ Brachy ($+T$) and $\frac{1}{4}$ tailless (Tt^1). The total data (table 1, exp. 6) show an excess of tailless offspring, but this is due to the method of testing. Females being tested were considered to be $+t^1$ when they had produced at least one tailless offspring (in most cases more than one) by a Brachy male. Many females produced this result in their first litter and thus were not bred again. Such females obviously constitute a selected group in which the proportion of tailless should be in excess of the random expectation. Actually such "one litter" females produced 274 normal, 151 Brachy, 184 tailless. When this selected group is subtracted from the total offspring the totals become 490 normal, 234 Brachy, 227 tailless in good agreement with the expectation based on random segregation. The result shows that $+$ and t^1 disjoin normally in oögenesis and that viability and classification are not important as factors affecting the ratio.

Other $+t^1$ females (from $+t^1$ mothers or fathers) were tested later and gave the results shown in table 1, exp. 7. Those whose test was based on one litter gave 116 normal, 78 Brachy, 92 tailless, again an excess of tailless; those with more than one litter gave 80 normal, 59 Brachy, and 35 tailless. The latter result although based on few observations is not in good agreement with the 2:1:1 ratio obtained in the first experiment. We are unable to account for the excess of Brachys found in the second experiment.

The heterozygous males ($+t^1$) when likewise tested by Brachy females produced normal, Brachy and tailless offspring but the ratio was not 2:1:1. There was always an excess of tailless and a deficiency of Brachy

offspring and this was not due to the method of testing since large samples of offspring were obtained from each male. The total data (table 1, exp. 8) show 1820 normal, 378 Brachy and 1386 tailless. The ratio of normal (++ and $+t^1$) to combined Brachy and tailless ($T+$ and Tt^1) expresses the disjunction of T and $+$ in the Brachy mothers. This ratio is normal (1820: 1764). The ratio of Brachy to tailless, which expresses the disjunction of $+$ and t^1 in the test males and should thus be 1:1, is instead about 1:3.7. The total ratio, however, has little significance since it represents an average of the ratios from 42 different $+t^1$ males, and there are large and significant differences among the ratios produced by different males. These differences are discussed in a later section.

It is evident from these results that tailless animals of Line 29 are Tt^1; that when these are crossed with normal (++) all progeny inherit either T or t^1, never both or neither; that females heterozygous for t^1 (Tt^1 or $+t^1$) transmit t^1 to half their progeny, while tailless males or males heterozygous for t^1 transmit t^1 to many more than half of their progeny.

CROSSES OF TAILLESS BY BRACHY

These conclusions were tested by crossing tailless with Brachy reciprocally. Matings of tailless females by Brachy males (table 1, exp. 4) produced but few offspring but these consisted of equal numbers of normal, Brachy and tailless. The cross may be formulated thus:

$$Tt^1 \times T+$$

TT	Tt^1	$T+$	$+t^1$
dies	tailless	Brachy	normal

The reciprocal cross (exp. 5) produced the same three classes but in proportions that differed significantly from 1:1:1. The tailless class exceeded the Brachy class by about 4.5:1; this means that about four and a half times as many offspring received t^1 from the father as received T. Here again there was great variability among the individual tailless males, a point that will be discussed later.

TESTS OF EXTRACTED TAILLESS

Tailless offspring from experiments 4, 6 and 7 (table 1) were bred together to test whether they were of the same genetic constitution as the original tailless animals. All such matings produced only tailless progeny (table 1, exp. 1b) showing that the balanced lethal condition (Tt^1) had been reconstituted by segregation. In one case a tailless male from experiment 5 when crossed with a tailless female from stock produced normal, Brachy and tailless offspring and was shown to be $+T$. In this case, as in

the three exceptional tailless animals from experiment 3, the tailless animals proved to be Brachys with extreme tail reduction. It had been shown by DOBROVOLSKAIA-ZAWADSKAIA, KOBOZIEFF and VERETENNIKOFF (1934) that certain Brachy stocks produced many tailless animals of genotype $+T$. The very small amount of overlapping between tailless and Brachy in our experiments is probably due to continued backcrossing of our stocks to the Bagg albino normal strain which has reduced the variability in expression of the Brachy mutation.

ABERRANT RATIOS FROM TAILLESS MALES

In table 2 are listed the progenies produced by the 20 tailless males (Tt^1) which, when crossed with inbred Bagg albino females, produced 11 or more offspring each. This table includes 711 of the 735 offspring shown in table 1, exp. 3. The remaining young came from males that sired too few young to reveal a significant ratio of normal to Brachy. In table 2 it is clear that

TABLE 2

Offspring from individual tailless (Tt^1) males mated with normal females.

MALE NO.	OFFSPRING		TOTAL	LITTER SIZE	APP. RATIO
	NORMAL	BRACHY			+: BRACHY
3290	97	8	105	8.8	12:1
3479	83	13	96	8.0	6:1
3791	61	8*	69	6.9	8:1
3167	38	13	51	7.8	3:1
38580	41	2	43	6.1	20:1
34818	38	2*	40	6.7	19:1
34259	34	2	36	**	17:1
2220	27	8	35	7.0	3:1
34815	25	7	32	8.0	4:1
2666	25	2	27	**	12:1
3539	26	1	27	9.0	26:1
35080	24	—	24		
2539	19	2	21		
3228	19	1	20		
3789	17	2	19		
2783	15	—	15		
2516	11	4	15		
3168	13	1	14		
3882	8	3	11		
4767	7	4	11		

* One was tailless but genetically $T+$.

** Litter size observations not comparable.

no single male produced the ratio of 1 normal:1 Brachy which was expected. All produced an excess of normals. The ratios varied from about 3:1 to over 20:1, and there was no modal ratio about which the frequencies tended to cluster. The variations appear to be associated with the indi-

viduality of the male and not with the females with which he was mated. Most of the females were taken from the 40th–50th brother-sister generation of the Bagg albino stock.[2] As an additional precaution the females were bred in succession to various of the males, so that the progenies are comparable in respect to mothers' genotypes.

The variation among individual males appears not to depend upon the extent to which they had been backcrossed to the Bagg albino stock. Males 35080, 34815, 34259, 34818 were from the original stock before backcrossing to the Bagg stock had begun; males 3290, 3882, 3789, and 3791 (the last two being full brothers) were from the fourth backcross to the Bagg stock; others were from intermediate backcross generations.

The variations are not due in any important degree to errors in classification. The evidence for this is the result of progeny testing 134 F_1 normals from this cross (p. 590), all of which proved to be $+t^1$. In the case of several of the tailless males listed in table 2, a fair sample of the normal progeny were tested and no exceptions to this rule were found. Thus, of the 97 normal-tailed offspring of ♂ 3290, which gave a very aberrant ratio, 35 (24 ♀♀ 11 ♂♂) were tested by Brachy; all proved to be $+t^1$; from ♂ 3167, which gave a less aberrant ratio, 19 F_1 normals were tested and all proved to be $+t^1$; from ♂ 3479, 12 F_1 normals were tested and found to be $+t^1$; from ♂ 2783, which gave only normal offspring, 8 F_1 normals were tested and all were $+t^1$.

Differential viability before birth of the two classes of offspring (normal and Brachy) is likewise not an important factor. The litter size in general was high (about 7.5), and did not differ significantly (table 2) between those progenies with a low and those with a higher proportion of the deficient class (Brachy).

To determine whether such aberrant ratios occurred only in the crosses of tailless males by normal females, some of the same males were tested also by Brachy females, which had been derived from backcrosses to the Bagg albino stock. Again the females were bred in succession to the different males so that in this respect the litters are comparable. A comparison of the ratios thus obtained with those from tailless by normal is given in table 3.

The ratios of normals to Brachys in each cross measure the segregation of t^1 from its allele in the father. In general the data from individual Brachy mothers are inadequate for close comparison of such ratios. There is however a general similarity between the ratios given by the same male. The greatest departure occurs in the progenies from 3479 in which the ratios are about 6:1 from normal mothers, and about 2:1 from Brachy

[2] We are very grateful to DR. E. C. MACDOWELL for supplying us with a number of such breeding females at a time when our stocks were low.

TABLE 3

Comparison of progenies obtained from tailless males when bred to normal (++) and Brachy (+T) females.

TAILLESS MALE	PROGENY FROM NORMAL FEMALES		PROGENY FROM BRACHY FEMALES		
	NORMAL	BRACHY	NORMAL	BRACHY	TAILLESS
2666	25	2	18	3	13
2783	15	—	3	—	5
3167	38	13	8	4	17
3290	97	8	8	2	6
3479	83	13	44	22	33
3539	26	1	6	—	13
34259	34	2	18	—	13

mothers. A longer series of such tests will be necessary to decide the question. At present it can only be said that most of the males gave consistent results with the two types of females, indicating that the aberrant ratios are not peculiar to the progeny from normal mothers and that there are individual differences among the males in the transmission of t^1. It will be recalled (table 1) that the ratio of normal to Brachy from all tailless males bred to normal females was about 7:1 (647:87), while the ratio of normal to Brachy from all tailless males bred to Brachy females was about 5:1 (231:48). The similarity may be in part fortuitous and due to the particular samples of males tested, but it shows at least that the essential feature of the aberrant ratios from various males is the relative frequency of transmission of t^1.

"CLUSTERS" OF OFFSPRING FROM TAILLESS FATHERS

Among the progenies from tailless males bred to normal females, it was noticed that the less frequent type of offspring (the Brachy type) had a peculiar distribution in the various litters from the same male. Thus ♂ 3290 sired 12 litters consisting of 97 normal and eight Brachy. Only four of these litters contained a Brachy offspring (one litter contained three, two litters contained two each, one litter contained one), a total of eight Brachys among 39 offspring; while eight litters comprising 66 offspring contained no Brachys at all. It is obvious that the chance of occurrence of a Brachy offspring in the total progeny is 8/105 or .076, whereas the actual frequency of Brachys in one litter of nine was three, or .333. The standard error of a ratio of .076 in a population of nine is .088. The deviation from expectation for the frequency of Brachys in this litter is therefore $.333 - .076 = .257 \pm .088$, which is a significant departure. Similar departures were found in other litters. The statistical significance of the occurrence of such clusters was therefore investigated in litters from the four males which produced the largest numbers of litters. The method chosen

was to calculate χ^2 and P for the ratio of the aberrant litter in comparison with the total ratio from that male in all litters. For example, litter a from ♂ 3290 (table 4) contains six normals and three Brachys; the total progeny of ♂ 3290 contains 97 normals and eight Brachys. Comparison of these two ratios by the method of BRANDT and SNEDECOR (cf. MATHER 1938 p. 23) yields $\chi^2 = 9.2477$, $n' = 1$, $P = <.01$; that is, in less than one percent of the cases would a departure as bad or worse than 6/9 be found by chance alone, where 97/105 is expected.

The results of these calculations are given in table 4.

TABLE 4

Distribution of normal and Brachy offspring within individual litters from four tailless males mated with normal females, together with the probability (P) of the deviation found for the most aberrant litters.

LITTER	♂ 3290			♂ 3479			♂ 3791			♂ 3167		
	NORMAL	BRACHY	P	NORMAL	BRACHY	P	NORMAL	BRACHY	P	NORMAL	BRACHY	P
a	6	3	<.01	6	3	.07	5	3	.02	3	4	.05
b	9	2	.16	7	3	.11	4	2	.09	4	4*	.09
remaining litters	82	3		70	7		52	3		45	10	
Totals	97	8		83	13		61	8		52	18	

* From normal female, not Bagg albino.

The most aberrant litters show departures from the total ratio for the particular male that are clearly significant, while several other values of P are near the 5 percent point. Since significant departures occur in several cases we may ask what is the chance that sampling alone should lead to a coincidence of such values of P as are shown in table 4. As FISHER (1938 pp. 104–5) has shown, a composite test of significance of several values of P is made possible by the fact that the sum of a number of values of χ^2 is itself distributed in a χ^2 distribution. Since for two degrees of freedom ($n' = 2$) the natural logarithm of $P = -\frac{1}{2}\chi^2$, then $2\ (-\log_e P) = \chi^2$.

Such a calculation applied to the four lowest values of P (one for each male) is shown below.

P	$-\log_e P$	degrees of freedom
.01	4.6052	2
.07	2.6593	2
.02 (.017)	4.0746	2
.05 (.046)	3.0791	2
	14.4182	–

$2(-\log_e)P) = \chi^2 = 28.8364 \quad n' = 8 \quad P = <.001$

There is thus less than once chance in a thousand that the coincidence of these values of P is due to sampling.

If to this we add the combined χ^2 values for the four next lowest values of P (.16, .11, .09, .09) we have still $\chi^2 = 46.7613$, $n' = 16$, $P = <.001$. This may be regarded as convincing evidence of the significance of the cluster phenomenon.

TABLE 5

Progenies of normal-tailed males heterozygous for t^1 when tested by Brachy females (T+). Only progenies of 37 and over have been included.

MALE NO.	OFFSPRING			TOTAL	MEAN LITTER SIZE	RATIO TAILLESS TO BRACHY
	NORMAL	BRACHY	TAILLESS			
2420	69	4	41	114	7.6	10.2
2519	83	13	61	157	7.5	4.7
2520	71	14	47	132	8.2	3.4
2786	27	3	25	55	7.9	8.3
2931	20	6	14	40	6.6	2.3
2946	53	13	35	101	7.2	2.7
2968	37	3	28	68	7.6	9.3
3317	17	—	21	38	7.6	—
3318	86	17	58	162	9.0	3.4
3362	32	—	28	60	8.6	—
3464	40	17	31	88	7.3	1.8
3466	62	24	31	117	8.4	1.3
3471	59	18	45	122	8.1	2.5
3598	98	7	78	183	9.1	11.1
3642	115	22	75	212	8.6	3.4
3644	80	34	61	175	9.2	1.8
3685	15	1	21	37	7.4	21.0
3686	26	11	16	53	4.8	1.5
3761	41	12	33	86	8.6	2.7
3764	35	12	29	76	7.6	2.4
3860	83	18	77	178	8.9	4.3
3861	48	16	33	97	8.8	2.1
3863	27	5	17	49	12.1	3.4
3864	66	18	56	140	9.5	3.1
3865	46	12	29	87	9.7	2.4
4006	23	3	11	37	9.2	3.7
4010	54	15	45	114	9.5	3.0
4272	15	5	17	37	9.2	3.4
4273	25	10	20	55	7.9	2.0
4429	70	4	65	139	8.2	16.0
4474	52	5	38	95	8.4	7.6
4684	57	6	62	125	8.3	10.3
4782	31	4	17	52	6.5	4.2
4796	53	12	51	116	8.9	4.2
5081	33	5	23	61	10.1	4.6
5086	21	3	23	47	8.1	7.7
Totals	1770	372	1362	3504	8.3	3.7

The clusters, it should be noted, consist of groups of litter mates of the rare type, in this case those receiving T from the father. They have been found only among litters from fathers that transmit t^1; on the other hand, by the methods used such clusters can be detected and subjected to statistical tests only where one class of offspring has a low frequency and where large total progenies are available, as in several of the matings of normal females by Tt^1 males. The detected cases are thus due, at least indirectly, to the presence of t^1 in the father. These cases suggest that groups of sperm of the same genotype with respect to T remain together during ejaculation. Further discussion is deferred until the data from t^1+ males have been examined.

VARIATIONS IN RATIOS FROM t^1+ MALES

The progenies produced by 36 males heterozygous for t^1 when mated with Brachy females are shown in table 5. This table includes 3504 of the 3584 progeny from such matings as given in table 1, exp. 8. The remaining 80 offspring occurred in progenies yielding less than 37 individuals each; from these reliable ratios cannot be obtained and they have therefore been omitted.

From the cross t^1+ by $T+$, a ratio of 2 normal:1 Brachy:1 tailless is expected. The total results in table 5 show that the numbers of normals (1770) and of mutants (1734) are about equal; thus that segregation of T and $+$ in the mothers is normal, and that these phenotypes are as viable as normal and are accurately separable from normal. The ratio of Brachy to tailless, however, is not 1:1 either in the total or in any single progeny. This ratio expresses the segregation of $+$ and t^1 in the father; the offspring receiving t^1 are always in excess. The ratios of Brachy to tailless vary in the progenies from 1:1.3 to 0:28, and there appears to be no single modal ratio about which the frequencies cluster. The frequency distribution of the ratios is as follows:

ratio	frequency
1.0–1.9	4
2.0–2.9	8
3.0–3.9	8
4.0–4.9	5
5.0–6.9	–
7.0–7.9	2
8.0–8.9	1
9.0–9.9	1
10.0– —	7
	—
	$n=36$

The largest numbers of ratios are those in the classes from about 1:2 to 1:4, but there are many ratios above 1:10. In an attempt to find reasons for this variability we compared, as in progenies from Tt^1 males, males with little and those with much of the blood of the Bagg albino stock to which the 29 stock was being backcrossed. No significant correlations were noted. The variations were not correlated with the average litter size of the progenies. Progenies with high ratios of Brachy to normal were of about the same size as those with low ratios as follows:

ratio	litter size
1–1.4	8.4
1.5–2.4	7.7
2.5–3.4	8.9
3.5–4.7	8.5
7–21	8.1

Differential mortality is therefore not a primary factor.

To test whether the variations in the ratios given by different males have a genetic basis the following experiments were made. From each of two tailless males which had given different ratios when crossed to normal, a series of sons was tested by crossing to the same group of Brachy females. The tailless males had been extracted from three backcrosses to the Bagg albino inbred stock and the Brachy females were from the third to sixth backcrosses to the Bagg stock. The results are shown in table 6.

The ratio of Brachy to tailless among the progeny of the sons of ♂ 3290 was 118:408 or about as 1:3.5; among the sons of ♂ 3167 it was 127:280 or about as 1:2.2. When the two ratios are compared by χ^2, P is less than .01, so they are significantly different. The ratios produced by the sons vary in the same direction as the ratios produced by the respective fathers.

A fact that is just as evident is the heterogeneity among the sons of the same male in respect to the ratio of Brachy to tailless among their progeny. The significance of this heterogeneity has been tested for each group of sons by the method of BRANDT and SNEDECOR. For the ten ratios of the sons of ♂ 3290, $\chi^2 = 16.84$, $n' = 9$, and $P = .05$; for the seven ratios from sons of ♂ 3167, $\chi^2 = 16.59$, $n' = 6$ and $P = .01$. The chance that the variations among the ratios produced by a group of brothers are due to sampling errors is very small indeed.

This selection experiment is being carried further. Sons ($+t^1$) of ♂ 3598, which gave the lowest proportion of Brachys, are being tested by Brachy females for comparison with sons of ♂ 3471, which gave a higher proportion of Brachys. To date these tests have produced the following results (table 7).

TABLE 6

TAILLESS ♂	F_1 NORMAL OFFSPRING BY BAGG ALBINO ♀		RESULTS OF TESTING F_1 NORMAL MALES BY BRACHY FEMALES			
	NORMAL	BRACHY	♂ NO.	NORMAL	BRACHY	TAILLESS
			3598*	98	7	78
			3761	41	12	33
			3764	35	12	29
			3860	83	18	77
3290	97	8	3861	48	16	33
			3863	27	5	17
			3864	66	18	56
			3865	46	12	29
			4006	23	3	11
			4010	54	15	45
			Totals	**521**	**118**	**408**
			3464	40	17	31
			3466	62	24	31
			3471	59	18	45
3167	38	13	3642	115	22	75
			3644	80	34	61
			3685	15	1	21
			3686	26	11	16
			Totals	**397**	**127**	**280**

* F_1 normal male from ♂ 3290 by Brachy (Bagg) female.

TABLE 7

$+l^1$ ♂	OFFSPRING BY BRACHY ♀			TESTS OF $+l^1$ SONS BY BRACHY ♀			
	NORMAL	BRACHY	TAILLESS	$+l_1$ ♂	NORMAL	BRACHY	TAILLESS
				4782	31	4	17
				4796	53	12	51
3598	98	7	78	4684	57	6	62
				4474	52	5	38
				4429	70	4	65
				Totals	**263**	**31**	**233**
3471	59	18	45	4273	25	10	20
				4272	15	5	17
				Totals	**40**	**15**	**37**

The results are rather meager but again the sons of the "low ratio" male give low ratios, and those of the "higher ratio" male give higher ratios. The difference between the total ratios from sons of ♂ 3598 and of ♂ 3471 is significant ($\chi^2 = 16.08, n' = 1, P = < .001$). The five sons of ♂ 3598

are now substantially homogeneous with respect to the ratios of Brachy to tailless which they produce ($\chi^2 = 7.24$, $n' = 4$, $P = .12$).

It is thus possible that the differences in the ratios are genetically determined by factors in addition to those at the *T* locus. These variations however are clearly of a secondary nature. The fact of primary importance is that ratios from *all* males containing t^1 are abnormal in the same direction; that is, t^1 is always transmitted more frequently than its alternative condition whether this is *T* or +; it is only the *degree* of excess of t^1 transmission that is apparently controlled by other factors. The source of the primary disturbance associated with t^1 has therefore been investigated in one further experiment.

In the case of the tailless males (Tt^1) it was shown that the aberrant ratios were due to the transmission of t^1 to a much larger fraction of the offspring than its alternative condition *T*. This was proved by progeny testing the normal offspring of matings of tailless males by normal females. All such tested normal offspring (which occurred in much higher proportions than expected) were shown to contain t^1. If the $+t^1$ males likewise transmit t^1 more frequently than +, then when $+t^1$ males are bred to pure normal females, two classes of progeny should be produced in *unequal* numbers, that is, $+t^1$ and ++, each having a normal phenotype. Moreover, if the $+t^1$ male has been previously tested by Brachy, the ratio among his two classes of normal progeny should correspond to the ratio found among his Brachy and tailless progeny from the Brachy test. This is readily seen if we arrange these two test crosses in the form of a checkerboard as shown below.

		Brachy ♀ eggs		normal ♀ eggs
		+	*T*	+
F_1 normal ♂ sperm	+	++ (*n*)	+ *T* (Brachy)	++ (*n*)
	t^1	+ t^1 (*n*)	t^1T (tailless)	+ t^1 (*n*)

The zygotes below the dotted line arise from the t^1 sperm; those above from the + sperm; therefore the two types of normal tailed offspring should bear the same ratio as the Brachy offspring bear to the tailless ones. The two types of normals can be distinguished only by progeny testing (crossing with Brachy). A few such tests have been made.

Thus ♂ 3471 gave when crossed with Brachy: 59 normal, 18 Brachy and 45 tailless (table 5). This male was then crossed with normal (inbred Bagg albino) females and produced 52 normal offspring. Eighteen of these normals (2 ♂♂, 16 ♀♀) were tested by Brachys. Twelve of them proved to be $+t^1$ (producing at least two tailless offspring); six of them proved to be ++ (producing no tailless among at least 15 offspring). The ratio of normals with t^1 to normals without t^1 is thus 2:1 as compared with a ratio

of tailless to Brachy of 2.5:1 (45:18). Another male (3598) tested by Brachy gave a more extreme ratio of 98 normal, seven Brachy and 78 tailless. Nineteen normal offspring of this male (6 ♂♂, 13 ♀♀) were tested. Eighteen of them proved to be $+t^1$, one proved to be $++$. This ratio is comparable with the ratio of 11.1:1 (78:7) among the tailless and Brachy offspring of ♂ 3598, when he was directly tested by Brachy. From other males which had given extreme ratios, seven F_1 normals (from crosses of such males with Bagg stock females) were tested; all proved to be $+t^1$. As controls ten normals from F_1 *females* ($+t^1$) were tested. Here the transmission of t^1 was shown by direct tests to be normal (p. 591) and equal numbers of $++$ and $+t^1$ offspring are to be expected. The numbers found were six $+t^1$ and four $++$. These data are shown in table 8.

TABLE 8

Distribution of $+t^1$ and $++$ genotypes among normal progeny of $+t^1$ males and females as revealed by progeny tests.

PARENT	NO. OF OFFSPRING TESTED	$+t^1$ OFFSPRING	$++$ OFFSPRING
♂ 3471 $+t^1$	18	12	6
♂ 3598 $+t^1$	19	18	1
other ♂♂ $+t^1$	7	7	—
other ♀♀ $+t^1$	10	6	4

The tests while not conclusive in themselves tend to confirm our previous conclusion that a primary cause of the altered ratios is an altered segregation ratio of t^1 from its alternative condition (either T or $+$) during spermatogenesis. Segregation in oögenesis is apparently not affected.

"CLUSTERS" AMONG OFFSPRING OF $+t^1$ MALES

Examination of the distribution of normal, Brachy and tailless offspring within the individual litters from crosses of Brachy females $+T$ by $+t^1$ males reveals many "clusters" of Brachy progeny, similar to those found among the offspring of tailless (Tt^1) males. For example, ♂ 3598 produced in 20 litters: 98 normal, 7 Brachy, and 78 tailless, a ratio of 1 Brachy to 11 tailless. Three of these Brachys occurred in one litter of nine (4 normal, 3 Brachy, 2 tailless), a ratio of 3 Brachy to 2 tailless, while the other nineteen litters contained 94 normal, 4 Brachy, and 76 tailless, a ratio of 1 Brachy to 19 tailless. A difference as great as occurred in this litter has a probability of less than one in a hundred. Such clusters occurred in at least one litter from 11 of the 36 $+t^1$ males which were tested.

The significance of such departures has been tested by the method of BRANDT and SNEDECOR, and those having a probability of .1 or less are shown in table 9. There are 24 litters from 11 different males which show

these low values of P. Of these, 18 litters show departures with a probability of .05 or below. Each of these may be considered significant.

TABLE 9

Distribution of offspring in single litters of 11 $+t^1$ males mated with Brachy females, together with values of χ^2 and of the probability, P, for the deviations in the ratio of Brachy to tailless found in the most aberrant litters, from the ratio found in all litters from the same male.

MALE NUMBER AND LITTER	NORMAL	OFFSPRING BRACHY	TAILLESS	χ^2	P
3318a	5	4		14.42	<.001
3318b	5	4	4	3.82	.050
3318c	3	2	1	3.45	.067
3318d	2	2	1	3.45	.067
3318 total	86	17	58		
3471a	7	4	1	7.03	<.01
3471 total	59	18	45		
3598a	4	3	2	18.84	<.001
3598 total	98	7	78		
3642a	5	3		10.09	<.01
3642b	3	4	2	6.19	.011
3642c	6	4	2	6.19	.011
3642d	4	2	1	3.21	.078
3642 total	112	23	75		
3644a	2	3		5.58	.019
3644b	11	3		5.58	.019
3644c	4	3	1	2.79	.096
3644 total	80	34	61		
3761a	3	3	1	5.24	.023
3761b	9	3	1	5.24	.023
3761c	3	3	2	3.19	.079
3761 total	41	12	23		
3860a	4	4		17.86	<.001
3860b	5	2	1	4.59	.035
3860 total	83	18	77		
3861a	4	5	2	5.58	.019
3861b	6	2		4.30	.041
3861 total	48	16	33		
3864a	5	3	1	5.90	.016
3864b	6	2	1	3.04	.085
3864 total	66	18	56		
4010a	4	5	2	9.11	<.01
4010 total	54	15	45		
4273a	5	4	1	5.88	.016
4273 total	25	10	20		

The probability of the coincident occurrence of these 18 clusters may be determined from the individual P values according to the method described above (p. 596), using as approximation .001 as the value of P in all cases in which P is less than .001 and .01 for values between .01 and .001. Thus:

No. of cases	P	$-\log_e P$	degrees of freedom
3	<.001	3(6.907) 20.721	6
3	<.01	3(4.605) 13.815	6
2	.011	2(2.207) 4.414	4
2	.016	2(1.833) 3.666	4
3	.019	3(1.661) 4.983	6
2	.023	2(1.470) 2.940	4
1	.035	1.050	2
1	.041	.892	2
1	.050	.693	2
18		53.174	36

$2(-\log_e P)=\chi^2=106.348 \quad n'=36 \quad P=<.001$

The probability that χ^2 exceeds expectation cannot be obtained from the usual tables which extend only to $n=30$. We use therefore the approximation $(\sqrt{2\chi^2}-\sqrt{2n-1})$ which in this case is significant and large (6.16). The value of χ^2 thus greatly exceeds that expected from random distribution of ratios. We may conclude that these clusters are thus not due to sampling errors but are clearly significant departures from normal segregation.

Each cluster consists of a group of individuals in the same litter which inherit the allele of t^1, in this case the type or normal allele. It is unlikely that all such clusters have been detected by the methods used, for they only become noticeable where one class of offspring has a low frequency in the total progeny and a much higher frequency within a litter. The variations in the ratios of Brachy to tailless from different males and the small number of offspring in a litter both tend to obscure the occurrence of such clusters, so the cases observed set only a lower limit to their frequency. The upper limit and the actual frequency remain unknown.

If, as has been suggested, such clusters are due to the production of groups of sperm with the normal allele (+) which stay together during ejaculation, then sperm with the other allele (t^1) may also occur in clusters. Since the latter type probably constitute the majority of sperm, clusters of these could not be detected by the methods used.

The litters containing clusters do not differ significantly in size from those not containing clusters, and normal (++, $+t^1$) and mutant ($T+$, Tt^1) offspring occur in equal numbers in both types of litter, showing that segregation of T and + in the mothers is normal and that phenotypic expression of these genes (their expressivity and penetrance) is not a factor producing the peculiar result. The deviations thus are probably due

to the distribution of t^1 and + offspring, the latter, at least, tending to occur in clusters.

The same cluster phenomenon was noted in the offspring of Tt^1 males and a subsequent paper will present data showing that it occurs also in the offspring of Tt^0 and $+t^0$ males. Since it has not been noted in other genotypes it is probably connected with the presence of either t^0 or t^1 in males and represents another similar effect of these apparently allelic mutations.

DISCUSSION

The evidence from breeding experiments with tailless mice of Line 29 and their descendants shows that two major mutations are involved, T and t^1. The dominant effects of T on tail length and its lethal effects on the embryo have been described by CHESLEY (1935). It has been shown to segregate normally in both sexes. The mutation t^1 is recessive, and when homozygous has a lethal effect on the embryo before implantation. These two in combination (Tt^1) produce the tailless phenotype. Since this type breeds true as a balanced lethal system, and no crossing over between T and t^1 has been observed, the mutations T and t^1 are assumed to be alleles. The mutation t^1 like the t^0 allele of T (CHESLEY and DUNN 1936), is now shown to segregate normally from the combinations Tt^1 and $+t^1$ in females, but in males it shows two major peculiarities: 1) heterozygous males of these genotypes always produce an excess of offspring with t^1; 2) in progenies from such males there appear clusters of offspring of one genotype with far greater frequency than expected by chance.

The first peculiarity was observed also in males carrying another allele of T, the mutation t^0. It was found (CHESLEY and DUNN 1936) that males heterozygous for t^0 ($+t^0$) regularly produced progenies in which the number of offspring receiving t^0 was either twice, or four times as great as the number receiving the normal allele. Thus six t^0+ males tested by Brachy ($T+$) females gave:

491 normal ($++$, $+t^0$)
297 tailless (Tt^0)
147 Brachy ($T+$).

Considering only the last two classes the number of those receiving t^0 from the father was almost exactly twice as great as those receiving the normal allele. Two other heterozygous males, tested by Brachy, gave:

159 normal ($++$, $+t^0$)
124 tailless (Tt^0)
34 Brachy ($T+$).

Here the ratio of the last two classes is about 4:1. It was suggested that a ratio of $2t^0$:1+ sperm would result if germ cells containing t^0 were to undergo one more equational division than those containing its normal

allele. Similarly, a 4:1 ratio would result from two additional equational divisions of t^0 cells. If extra equational divisions in spermatogenesis were the only cause of the altered ratios from males containing one of these alleles, then we should expect that the ratios of offspring receiving t^0 to those receiving + should always be a power of two, that is 2:1, 4:1, 8:1 . . . 2^n:1.

Ratios from $+t^1$ males

The ratios from $+t^1$ males have been found to vary from somewhat less than 2:1 up to 20:1 or more for different males, and there is no clustering of frequencies on the binomial terms as would be expected on the hypothesis of extra equational divisions of one type of germ cell. The same is true for ratios from Tt^1 males, although only a few of these gave sufficiently large progenies to constitute a test of this point.

This does not necessarily rule out the hypothesis of extra divisions, for other aberrations may cause the ratios to deviate from the basic ratios expected. Thus differences in viability between Brachy and tailless or overlapping variability between these classes might cause minor variations in the ratios of these types. Evidence has been given that neither of these factors is an important cause of the variations noted.

One further fact which must be taken into account is the tendency for males heterozygous for t^1 to produce clusters of one type of offspring. This introduces another factor causing variability in the ratios which may have an important effect. If ratios between two types of spermatozoa vary not by units consisting of single frequencies or individual sperm cells, but by groups, then the variance of the ratios is greatly increased. It can be shown for example that in litters of eight, in which the chance of occurrence of *a* or *b* is equal (normal segregation) and in which the proportion of *a* is free to vary from eight to zero by unit steps, the standard deviation of a normal distribution of such ratios is given by $\sqrt{2}$; where the proportion of *a* can take only the values 8, 6, 4, 2, 0 (groups of two) the variance of the ratios is $\sqrt{4}$; where *a* can take only the values 8, 4, 0 (groups of four), the variance of the ratios is $\sqrt{8}$. The increased variance due to the occurrence of grouping or clustering will thus tend to produce intergrading variation in ratios such as 1:2, 1:4, 1:8 which might be expected from additional equational divisions of one type of germ cell. In the frequency distribution of ratios of $+:t^1$ from different $+t^1$ males (p. 598) it is to be noted that while ratios of from 1:1 to 1:5 occur frequently, there are no values between 1:5 and 1:6.9, followed by a few values from 1:7 to 1:9.9 and several around higher values. The discontinuity may be due to sampling, especially since several males selected from a "low-ratio" father (♂ 3598) are included. The data, however, do not exclude the possibility

that the ratios belong to a series based on powers of two, when the variance due to clustering is taken into account. Further analysis is deferred until a larger sample of ratios given by males from a single father is available.

The occurrence of "clusters" of offspring receiving an allele of t^1 (either T or $+$) from the father suggests some form of association among this type of spermatozoa. The most obvious interpretation would be that groups of spermatozoa formed from adjacent spermatids remain together. But since all contain the same allele, such a distribution would be likely to occur only if such spermatozoa were to arise from one spermatid by further division. If the whole cluster were descended from one spermatid, all should contain either the X or the Y chromosome and members of the cluster should thus be of one sex. This is not the case. Many of the clusters contain both males and females. Whether clusters of like-sexed individuals are more common than should occur by chance cannot be determined from the data on the few clusters detected. It is possible of course that the clusters containing both sexes arise from two smaller groups of sperm each alike in respect to sex chromosomes; and it is certain that not all T or $+$ offspring from Tt^1 or $+t^1$ fathers occur in clusters, so that sperm with these alleles may occur singly. Clusters from $+t^1$ males which are heterozygous for another mutant gene (Cc^a) contain both C and c^a individuals so that it is unlikely that clusters arise from reduplication of spermatids. An adequate interpretation of the peculiar cluster phenomenon cannot be given from the data in hand and must await the results of cytological study.

Cases similar to the above have not come to our attention. The sexual difference in segregation ratios is reminiscent of the cases reported in several species of plants in which mutant conditions are transmitted with different frequency through the egg and through the pollen, but in these cases the failure of the gametophyte (pollen tube) containing the mutant factor is usually responsible. In our case, the gametes containing a lethal are recovered with much *higher* frequency than those with the normal allele.

The cluster phenomenon also appears not to have been noted heretofore. GRÜNEBERG and HALDANE (1937) have devised methods for detecting such occurrences in single gene segregations and have studied from this point of view the segregation in backcross experiments of five single, independent gene differences and of sex in 1304 litters of mice containing 7550 offspring. In only one out of 13 experiments did the χ^2 test reveal any significant disturbance in the segregation ratios. Although the authors suggested that inequality in numbers of gametes containing members of a pair of unlike alleles might cause such a departure, they were inclined

to regard the single case detected as due to the linkage of a lethal or lethals with one of the alleles whose segregation was followed. Had "clusters" of offspring containing one of the five segregating genes occurred, they would have been detected. "Clustering" is thus not a normal occurrence in the mouse.

From cytological evidence, HERTWIG (1933) has assumed that additional equational divisions may occur normally in spermatogenesis in the cat and in man. His conclusions are based on measurements of nuclear size in spermatocytes and spermatids. He distinguishes four size groups of nuclei with volume ratios of 8:4:2:1, instead of the three groups expected from the occurrence of two maturation divisions. His interpretation is that in cat and man the secondary spermatocytes undergo one additional equational division, resulting in an additional reduction in the size of the nuclei. In rats which normally have only two meiotic divisions in spermatogenesis, treatment with Prolan yields four size groups of nuclei and it is assumed that an additional equational division results from such treatment. Recently HERTWIG (1938) has referred to two additional proofs for the existence of three meiotic divisions in cat and man: measurements of plasma volumes of primary spermatocytes and spermatids, the ratios of which are 8:1, and the existence of three different sizes of metaphase plates in spermatogenesis. Although these observations have not yet been confirmed, the possible occurrence of an additional equational division in spermatogenesis in some mammals is of interest in view of our suggestion, based only on genetical evidence, that additional equational divisions in the mouse may occur in males which are heterozygous for either t^0 or t^1.

SUMMARY

1. Breeding experiments with a true-breeding line of tailless mice (Line 29) have shown that the animals of this line are heterozygous for two lethal mutations; namely, T (lethal to embryos at 11 days) and t^1 (lethal before implantation). Interaction between these mutations produces the tailless phenotype Tt^1. Since no crossing over occurs between T and t^1 the genotype Tt^1 constitutes a balanced lethal line and T and t^1 are assumed to be alleles.

2. Tailless females when outcrossed with normal $(++)$ or with short-tailed (Brachy) males $(T+)$ show normal segregation for the dominant T and the recessive t^1.

3. Tailless males when crossed with normal or with Brachy females segregate for T and t^1 but offspring inherit t^1 with much higher frequency than T, and those inheriting T tend to occur in groups or clusters, forming significant departures from normal segregation.

4. Normal-tailed females heterozygous for t^1 transmit t^1 to half their

progeny. Crosses of such t^1+ females by Brachy $(+T)$ males produce the expected ratios of .50 normal:.25 Brachy:.25 tailless.

5. Normal-tailed males heterozygous for t^1 always transmit t^1 to more than half their progeny. Crosses of such males $(+t^1)$ by Brachy $(+T)$ females produce ratios varying for individual males from about .5 normal :.2 Brachy:.3 tailless to .5 normal :.025 Brachy:.475 tailless. The progeny inheriting the allele of t^1 tend to occur in clusters within single litters.

6. These results are interpreted as due to an effect of the t^1 allele on segregation, possibly through causing additional equational divisions in spermatogenesis.

7. The "cluster" phenomenon is probably due to association among spermatozoa containing the allele of t^1 but cannot be given a final interpretation from present evidence.

8. Both of the latter effects on segregation occur also in the presence of another mutant factor, t^0, which acts as an allele of T and t^1.

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STUDIES ON THE TRICOLOR PATTERN OF THE GUINEA PIG[1]. I. THE RELATIONS BETWEEN DIFFERENT AREAS OF THE COAT IN RESPECT TO THE PRESENCE OF COLOR

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INTRODUCTION

SPOTTING patterns serve as one approach in the study of gene action. A superficial character, such as pigmentation, presumably is closer to primary gene action than are the more fundamental aspects of embryonic development. The spotting problem, concerned with the localization of color, is to be distinguished from the pigment problem concerned with the quality and intensity of color. The present paper is a descriptive study of the spotting pattern of the guinea pig, a pattern which presents features of interest in the study of color localization. This pattern is highly irregular, practically every individual being unique. Yet, underlying this great irregularity is a regularity to be seen in composite patterns.

GENETICS OF THE TRICOLOR PATTERN

The tricolor pattern can be analyzed into two components: (1) the pattern of color and white and (2) the pattern of black and yellow. Only the former will be considered in the present paper.

Colored areas on a white background constitute the white spotted pattern. As the amount of color increases, white occurs only at the breaks between spots or at the extremities (nose, forehead, feet, belly). The appearance is of colored spots draped over the sides of the animal but frequently too small to overlap or reach the extremities. Individuals exhibit great asymmetry in extent and even in occurrence of these spots.

The genetics of the white spotted pattern in the guinea pig has been considered at length in a previous paper (WRIGHT and CHASE 1936). The factors can be classified into four categories. First, there is a major pair of alleles, *S*, *s*, with *S* usually incompletely dominant over *s*. Second, there is a multiplicity of genes with additive effects, showing no dominance or factor interaction, and capable of shifting the median grade of *ss* from 10 to 97 percent white, and *SS* from self to a little white. These modifiers

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are largely quantitative relative to the coat as a whole, but EATON (1928) described one localization tendency in an inbred strain (a branch of strain 2) in which 22 percent of the animals have colored nose spots in contrast with the usual white of this region. Third, there is a great amount of non-hereditary variability, accounting for about 58 percent of the variance in a random bred stock of constitution *ss*. In an isogenic strain, with median grade near 50 percent, this variability gives a range from a trace of white to 100 percent white. A small part of this variability is common to littermates, about eight per cent in the isogenic strain, of which about half is due to the age of mother (increased amount of white in offspring with increased age of mother). To what extent this non-hereditary variability, largely not common to littermates, is common to the individual as a whole is a question which will be considered here. Fourth, there is a sex difference, the females tending to have a little more white than males.

The pattern is of a type common in mammals. It is somewhat more irregular than the pattern of the spotted house mouse (DUNN and CHARLES 1937) or Holstein cattle (DUNN, WEBB, and SCHNEIDER 1923) and much more irregular than that of the Dutch rabbit (CASTLE 1925). It is at the opposite extreme from the highly regular pattern of the hooded rat (CASTLE and PINCUS 1928). The guinea pig has more non-hereditary variability than any other spotted mammal that has been studied.

In at least one case in mammals, quality of color affects the amount of white spotting. LITTLE (1917) found that dominant yellow of mice (gene A^y), lethal when homozygous, reduces the amount of white in spotted animals. This situation has been studied further by DUNN, MACDOWELL, and LEBEDEFF (1937) and found to be an interaction effect with what they call the *m*(*W*) complex of modifiers for variegated spotting. There was no interaction effect either with the piebald factor (*s*), the "k" modifiers of piebald spotting, or with the variegated factor (*W*) in the absence of the *m*(*W*) complex. Also there was no effect of the alleles of A^y (*A* and a^t). Further, it should be noted that the white spotting of these yellow mice was distributed in coarse spots (pied type) rather than of the finely spotted or variegated type. No cases of this sort have yet been clearly demonstrated in the guinea pig.

THE DESCRIPTIVE PROBLEM

The questions concerning gene action in relation to the spotting pattern of the guinea pig are not likely to be solved without the cooperation of several methods of attack. But more precise descriptions of the actual situation in the guinea pig are important for more clearly formulating the problem. The present paper is concerned with such a description, in particular the following points:

1. The regularities underlying the apparent irregularity of the spotted pattern.

2. The interrelationships between different areas of the coat with respect to occurrence of color or white within inbred stocks. This bears only on the nature of the non-hereditary factors.

Average pattern of color and white

Definite points in the coat were selected and their location was indicated on a diagram on transparent tracing cloth. The presence or absence of color was tabulated for each selected point in the birth record of all animals of the strains studied. Figure 1 shows the percentage frequency of color for 30 points in 157 animals of strain DS. This strain derives its

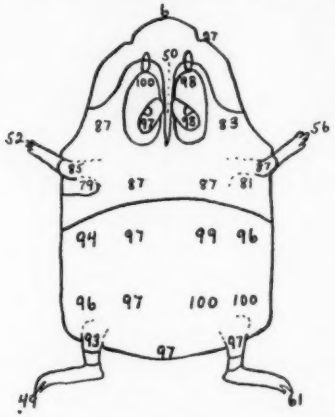


FIGURE 1.—DS. Color frequency (percent) at given points.

spotting (*ss*) from the inbred strain 2, described later, the median grade of white of which is at 94 percent. This strain was backcrossed for three successive generations to a self colored strain (D), constitution *SS*. Homozygous spotted (*ss*) were then extracted as described by WRIGHT and CHASE (1936). These extracted spotted have very much less white (median 6.5 in males, 12.0 in females) than strain 2 because of modifiers from D.

Figure 2 is a composite of 405 animals in strain 35D, derived from one mating in the 22nd generation of brother-sister mating. In this case 81 points were used. This strain is a medium spotted type, median about 70 percent white and ranging from a trace to all white.

Figure 3 is a composite of 741 animals in strain 13E, derived from one mating in the 18th generation of brother-sister mating. The same 30 points as in DS were used. This is the whitest of the inbred strains, median about 98 percent white and ranging from 65 percent to all white.

These three unrelated strains show an orderly progression in the disappearance of color. DS is usually white on the nose and lower lip, the nose showing color in only six per cent of the cases. The forehead and feet are about equally often colored and white. The rest of the coat is usually colored but a belt across the shoulders extending forward on the cheeks and ventral side of the head shows white more frequently than the posterior half of the body and especially the region about the ears and eyes. Streaks of white along the ventral sides of the legs frequently occur but are not shown in the diagram. Strain 35D exhibits a further decrease in color with frequency of color on the nose lower and on the feet much lower than in strain DS. The relatively low belt across the shoulders of

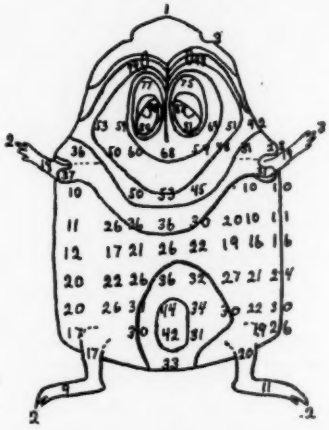


FIGURE 2.—35D. Color frequency (percent) at given points. Note: 36 percent at forehead point between ears.

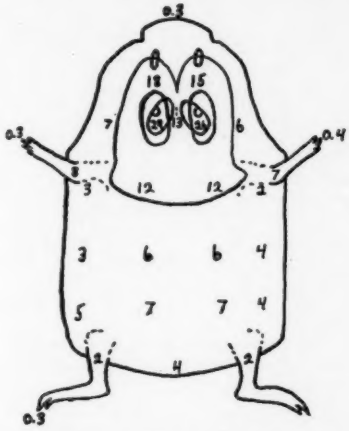


FIGURE 3.—13E. Color frequency (percent) at given points.

DS seems here to be more posterior. The extensive area of frequent color on the posterior half of DS is here restricted to a median dorsal area. In strain 13E the reduction of color has merely proceeded farther along the same lines. The ear regions of all three strains are relatively high in color frequency, and the ears tend to be the last to lose color.

In the high grade piebalds another method of describing the average pattern is appropriate. Since the color in these animals occurs in discrete spots, a point at the center of each spot can be rather accurately determined, and the frequencies of these points can be found in the various regions. In figure 4, based on the same animals of 13E as figure 3, each point indicates a region where these spot centers occurred. This method brings out certain aspects not brought out by the other method. The

distribution of centers is indicated by the contours and the relatively great frequency of color around the ears is demonstrated. Bilaterality is more clearly brought out by this method, especially in the loin regions in which figure 4 shows two high localities, whereas figure 2 shows a median high due to overlap of spots from either side. Figure 5 is the composite according to spot centers of 341 animals of strain 2. This is the strain referred to above as the source of *s* in strain DS. It traces from one mating in the 15th generation of brother-sister mating. In figures 4 and 5 the

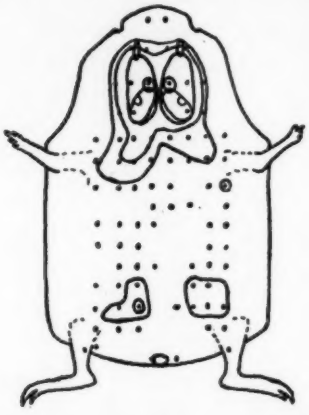


FIGURE 4.—13E. Spot center distribution (approximate). Points show regions in which any spot centers occurred.

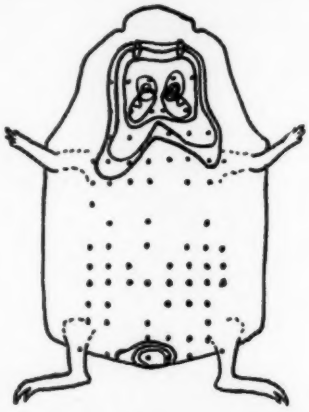


FIGURE 5.—2. Spot center distribution (approximate). Points show regions in which any spot centers occurred.

points show where spot centers occurred. None occurred on the feet or ventral side.

The orderly sequence exhibited in figures 1, 2, and 3 appears to be characteristic of strains of guinea pigs in general, with minor qualifications such as the more frequent occurrence of "tail" spots in strain 2 than in strain 13E. An orderly sequence has also been described for white spotting in mice (CHARLES 1938). It appears then that the hereditary modifiers for the most part are ones that affect the average amount of color, although hereditary factors affecting localization occur to a minor extent. In the stocks which have come under our observation, there are clearly no genes for particular spot centers or for particular white breaks.

Correlations within inbred stocks

In order to make an analysis of the nature of the irregularities of the individual piebald patterns, it is desirable to find the correlations between different areas of the coat in animals of an inbred stock in which all

hereditary factors responsible for the orderly differences in average extent of pattern, described above, have been eliminated. A strain whose average extent of colored spots is near the middle of the range is preferable. Strain 35D fits these requirements, being highly inbred and having the complete spotting range (median 67.8 for males, 73.1 for females). Of the 405 animals used (1926–1934) only four were all white. Thirty points (figure 6) were selected, put on a tracing cloth, and each animal was recorded separately as colored or white at each of these points.

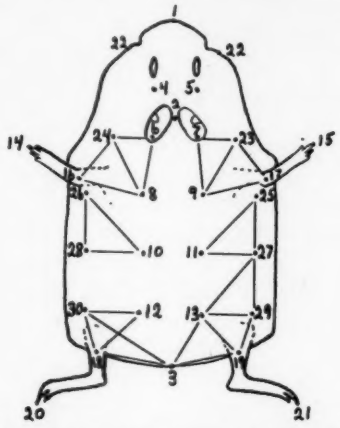


FIGURE 6.—Correlations between color frequencies of points tabulated in strain 35D. High correlations (+.40 to +.75) represented by lines.

The relationship between each point and every other point with respect to presence or absence of color was studied. As an example, the table for points 10 and 11 was:

10 \ 11	+	−	
+	41	45	86
−	49	270	319
	90	315	405

Numbers

10 \ 11	+	−	
+	.101	.111	.212
−	.121	.667	.788
	.222	.778	1.000

Proportions

The table means that when point 10 was colored, point 11 was colored in 41 animals or in 10.1 percent of the cases and white in 45 cases or 11.1 percent; when point 10 was white, point 11 was colored in 49 cases or 12.1 percent and white in 270 cases or 66.7 percent.

Six of the 30 points could not be used in obtaining correlations because of having frequencies of color below five percent (nose, feet, and lower lip). The remaining 24 points, ranging in color frequency from 10 to 81 percent, gave 276 four-fold tables. Two different correlation methods were tried, the tetrachoric and Yule's theoretical value for r. The results of the tetrachoric method were calculated by means of the "Computing Diagrams for the Tetrachoric Correlation Coefficient" worked out by PROFESSOR L. L. THURSTONE and LEONE CHESIRE of the University of Chicago.

After the calculations were completed an inconsistency was noted in connection with the points on the upper hind legs and "tail" (points 18, 19, 3). The correlation for 3 and 18 is +.91, for 3 and 19, +.84, but for 18 and 19, only +.29. With such high values for 3:18 and 3:19, it is impossible to have as small a value as +.29 for 18:19 if the table represents a dichotomy for each variable of an underlying normal frequency surface relative to color tendencies. As this indicated that the assumptions justifying the use of tetrachorics are not satisfied, it seemed best to use the simpler method.

The formula used is derived directly from the usual coefficient of correlation,

$$r = \left(\frac{\Sigma xyf}{n} - \bar{x}\bar{y}\right)\frac{1}{\sigma_x \sigma_y},$$

assuming two point variability in each case. The formula is as follows:

	+	−	
+	a	b	q_1
−	c	d	$1-q_1$
	q_2	$1-q_2$	1

$$r = \frac{a - q_1 q_2}{\sqrt{q_1(1 - q_1)q_2(1 - q_2)}}$$

$$\text{If } t = \frac{1}{\sqrt{q(1-q)}}, \quad \text{then } r = (a - q_1 q_2)t_1 t_2.$$

With Yule's theoretical value for r rather than the tetrachoric, no such inconsistency as that noted above arises; all coefficients are reduced in magnitude and in general proportionately. Table 1 consists of the 276 coefficients in strain 35D. The forehead (2) and eye (4, 5) points show a rather low correlation with each other. Apart from these, the points on each side fall into three groups within which there are high correlations (+.27 to +.75) but between which the correlations average much lower.

TABLE 1

Correlations between points in frequency with which they are pigmented in 405 animals of strain 35D from one mating in 22nd generation of brother-sister matings. Median amount of white 70 percent.

	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19	24	23	26	25	28	27	30	29
Forehead 2		−.04	+.19	+.15	+.14	+.18	+.17	+.13	+.04	+.01	−.07	−.01	+.17	+.09	−.06	−.03	+.14	+.13	−.04	.00	−.05	+.02	−.08	−.01
"Tail" 3			−.03	+.08	−.08	.00	−.09	+.04	−.06	+.04	+.37	+.45	−.04	−.01	+.62	+.57	−.02	.00	.00	−.01	+.11	+.08	+.43	+.39
Left "eye" 4				+.13	+.14	+.05	+.05	+.05	+.01	−.06	−.04	.00	+.07	−.03	.00	+.04	+.08	−.02	−.03	−.01	−.02	−.03	−.04	−.02
Right "eye" 5					+.07	+.33	+.03	+.06	−.05	−.01	−.05	+.02	+.04	+.07	−.07	+.09	.00	+.15	+.02	−.03	+.01	−.01	+.05	+.01
Left ear 6						+.14	+.46	+.18	+.06	+.06	−.05	+.01	+.29	+.12	−.07	−.05	+.48	+.19	+.06	+.09	+.03	+.03	−.04	−.01
Right ear 7							+.13	+.40	−.02	+.02	+.01	−.03	+.05	+.27	−.03	−.04	+.13	+.45	−.01	+.10	+.02	.00	+.02	−.04
Left shoulder 8								+.33	+.20	+.02	.00	−.01	+.69	+.21	−.05	−.01	+.67	+.20	+.19	+.10	+.08	+.01	+.02	+.05
Right shoulder 9									+.07	+.14	.00	−.04	+.25	+.67	+.01	−.07	+.23	+.61	+.08	+.32	+.06	+.09	−.06	−.05
Left side 10										+.32	+.22	+.13	+.35	+.12	+.02	+.04	+.07	+.02	+.47	+.22	+.56	+.16	+.18	+.17
Right side 11											+.22	+.36	+.02	+.23	+.09	+.08	−.03	+.04	+.11	+.48	+.23	+.68	−.15	+.33
Left hip 12												+.24	−.06	+.08	+.55	+.12	−.04	+.03	+.14	+.14	+.34	+.22	+.72	+.12
Right hip 13													+.03	+.04	+.17	+.61	+.05	−.07	+.06	+.16	+.11	+.40	+.17	+.70
Left upper fore leg 16														+.19	+.01	+.02	+.50	+.13	+.32	+.04	+.20	+.03	+.04	+.10
Right upper fore leg 17															+.06	−.01	+.14	+.56	+.06	+.43	+.05	+.19	+.12	+.01
Left upper hind leg 18																+.16	−.06	+.05	+.04	+.04	+.18	+.11	+.72	+.10
Right upper hind leg 19																	+.03	−.09	−.03	+.05	+.02	+.17	+.02	+.68
Left throat 24																			+.11	+.06	+.03	−.06	−.04	+.05
Right throat 23																	+.18		+.05	+.27	+.02	+.02	+.10	−.03
Left chest 26																					+.59	+.02	+.13	+.08
Right chest 25																			+.06		+.13	+.51	+.13	+.11
Left belly 28																							+.33	+.14
Right belly 27																					+.16		+.19	+.44
Left mamma 30																							+.11	
Right mamma 29																								

Correlations are higher across the midline of the back (average +.30) than across the mid-belly (average +.11). The dorsal points show their highest correlations with points ventrad (average +.53) or anteroventrad (average +.48). Between remote regions such as shoulder and hip of same side (average −.02), the correlations are essentially zero. Along the ventral surface, points on the same side show higher average correlations with each other than do the corresponding dorsal points with each other. The eye points (behind the eye itself), although only half as far from the ears as the latter are from the shoulders, show much lower correlations (average +.23) than would be expected from the distance. The high correlations (+.40 to +.75) are represented in figure 6. The apparent asymmetry in some respects probably has no significance.

In order to determine if similar relations held in other strains, the inbred strain 13E was tabulated for the 30 points. Here the median amount of white is 98 percent, the range, 65 percent to all white. Only thirteen points could be used because of color frequencies below five percent. The elimination from this cause of the ventral, "tail," and hind leg points limits considerably a direct comparison with the other results. With thirteen points there are possible 78 coefficients; these are presented in table 2. Comparable correlations tend to be slightly, but only slightly, lower in 13E than in 35D. Eye-ear correlation is considerably lower, however, and the forehead (2) point shows no correlation with any other point.

In figures 7, 8, and 9 the principal relationships between the points, as well as the relationships between the two strains, are brought out. Figure 7 shows the correlations between points along transverse lines. It brings out the relatively low correlation across the midline of the back especially in 13E. Shoulder-chest (+.255) is lower than other comparable correlations but the anteroventrad correlation with the throat (of course not shown in this figure) is high (+.64). Comparison of figure 8, giving the correlations between points along the ventral side, with figure 9, the correlations between points along the dorsal side, shows the less rapid falling off of correlations with distance in strain 35D. The latter figure has the eye point added. It is placed at twice its actual relative distance, which gives it a distance equal to the others. The close approximation of 13E and 35D, except for eye-ear, is obvious.

In strain 13E correlations were also obtained for presence or absence of spot centers. Right and left spot centers in the regions of the rump, side, shoulder, neck, head, and a median center on the forehead were used. Essentially zero correlations were obtained. All of these regions are independent of each other with respect to the non-hereditary factors alone responsible for variation in this stock.

ILJIN (1928) reported an extensive series of correlations relative to the

occurrence of white in various regions. Although he used the same correlation method, a comparison of the exact values is not possible since his correlations refer in the main to regions of occurrence of spot centers and also since the nature of his stock is not clear. His correlations were systematically higher than those reported here. Higher correlations would of course be expected if his stock were not genetically homogeneous (descended from a single mating after prolonged inbreeding). There is agreement, however, in the falling off of the correlations with increasing distance on the animal.

TABLE 2

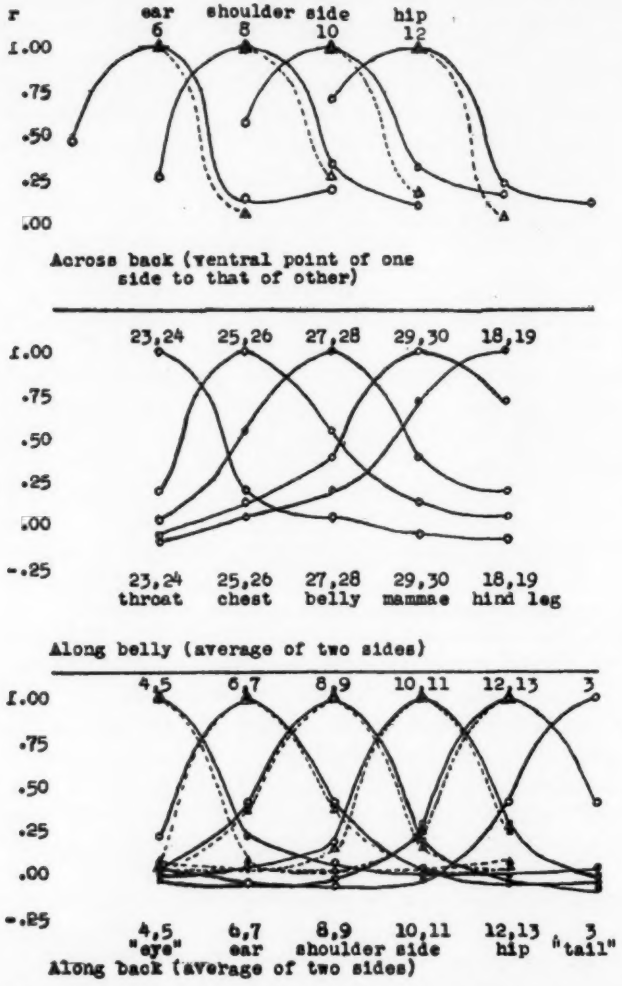
Correlations between points in frequency with which they are colored in 642 animals of strain 13E from one mating in 18th generation of brother-sister matings. Median amount of white 98 percent.

	2	4	5	6	7	8	9	10	11	12	13	16	17
Forehead 2		−.05	+.02	−.01	+.02	.00	−.04	−.04	−.03	−.01	−.01	−.06	.00
Left "eye" 4			+.02	+.07	+.03	.00	.00	+.02	−.04	+.05	.00	+.02	−.01
Right "eye" 5				−.01	+.07	−.03	+.04	+.04	+.05	+.02	+.07	−.02	.00
Left ear 6					+.04	+.40	+.08	−.01	+.07	+.01	+.05	+.30	+.09
Right ear 7						+.05	+.34	−.02	+.03	.00	+.05	−.04	+.24
Left shoulder 8							+.27	+.13	+.07	+.07	+.01	+.68	+.20
Right shoulder 9								+.04	+.14	+.12	−.01	+.15	+.65
Left side 10									+.17	+.20	−.03	+.32	+.06
Right side 11										+.02	+.27	+.07	+.16
Left hip 12											+.02	+.06	+.08
Right hip 13												+.06	−.02
Left upper fore leg 16													+.16
Right upper fore leg 17													

What points are brought out by these correlation studies of the spotting pattern? First, the determination of the pattern is probably during the embryonic stage when proportions are different from those of the newborn guinea pig. The relatively great size of the head could be responsible for the low correlations between the eyes and forehead and between these and all other points. The correlations between points along the belly further indicate the possibility of overlapping of streams of migrating potential pigment cells from dorsal regions. The second point concerns the comparison between 35D and 13E. The anteroposterior lengths of the spots (figure 9) are only slightly less in 13E but the dorsoventral lengths are considerably less (figure 7 and the very low frequencies of ventral points).

The principal deduction to be drawn from these correlations is that the pattern in animals of the same genotype is not determined for the animal as a whole, remote regions having zero correlations. The non-hereditary factors, alone responsible for the individual irregularities of an inbred stock, must thus act regionally rather than on the animal as a unit. This is in harmony with the low littermate correlations for grade of white, +.115 (35D) and +.018 (13E). Within regions, however, there is considerable correlation. Either a fairly large spot is present or there is no color at all. Very small spots (except on head and in caudal region) are rare. But

the occurrence of separate spots is almost purely a matter of chance. The final coat pattern is the result of the combination of the effects of



FIGURES 7 (above), 8 (center), 9 (lower).—Correlations represented across the back, along the belly, and along the back respectively. Solid lines, strain 35D; broken lines, strain 13E.

non-hereditary factors acting locally and of hereditary factors acting largely on the animal as a whole. Further discussion will be deferred until after discussion of the tortoise shell pattern in a subsequent paper.

SUMMARY

The genetics of the color-white pattern is reviewed.

Composite patterns of guinea pigs show symmetry and regularity for white spotting even though individual patterns are asymmetrical and unpredictable. In the main there is a regular sequence in the extent of color in such composite patterns from strains with little color to strains of self color. Hereditary factors determine the level of a strain with respect to the sequence. There are, however, minor differences in localization in the composite patterns that are hereditary.

Correlation studies of two inbred strains indicate that the non-hereditary factors involved in white spotting act locally rather than on the animal as a whole. All correlations between remote regions are substantially zero in such stocks.

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STUDIES ON THE TRICOLOR PATTERN OF THE GUINEA PIG.[1] II. THE DISTRIBUTION OF BLACK AND YELLOW AS AFFECTED BY WHITE SPOTTING AND BY IMPERFECT DOMINANCE IN THE TORTOISE SHELL SERIES OF ALLELES

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GENETICS OF THE TRICOLOR PATTERN

THE tricolor pattern of the guinea pig can be analyzed into two components: (1) the pattern of color and white and (2) the pattern of black and yellow. The former has been considered in the previous paper (CHASE 1939).

Pattern of black and yellow

The tortoise shell pattern consists of yellow hairs sprinkled lightly among black hairs in a few irregular, asymmetrical regions over the body. This is the typical pattern of family D, a highly inbred strain which never shows white. Occasionally a foot has a small area of segregated yellow at the tip. Large areas often appear to be solid black.

The tricolor pattern of black, yellow, and white, was a feature of the earliest scientific description of the guinea pig. According to CUVIER this variety was described by ALDROVANDUS about 1550 (CASTLE 1912). Compared with the tortoise shell, the tricolor has a tendency to greater segregation of black and yellow hairs (WRIGHT 1917), giving typically in addition to some brindling clear areas of black, yellow, and white. The individual irregularity of the black-yellow pattern is even greater than for the color-white pattern.

In 1916 IBSEN proposed the symbol e^p (partial extension of black) for the gene determining the tortoise shell pattern. Later (1919) he reported on the inheritance of the three alleles at this locus, E (self black) dominant over e^p and e (self yellow), and e^p dominant over e. The question of dominance in multiple allelic series is interesting and a quantititve study has been one of the objects in the present investigation.

No statistical analysis of the array of modifiers of this pattern has been made but the existence of such modifiers will be shown by differences in average number of yellow hairs in certain experiments.

[1] Submitted in partial fulfilment for the degree of Doctor of Philosophy at the University of Chicago.

[2] The author is grateful to PROFESSOR SEWALL WRIGHT of the University of Chicago for the suggestion of this problem, the use of pattern records, the facilities for breeding experiments, and the constant stimulating guidance throughout the work. The guinea pig colony has been maintained under a grant to the University of Chicago from the Rockefeller Foundation.

One modifier, however, has been a principal object of the present paper. This is white spotting. WRIGHT (1917, 1923) observed the relation between these two types of spotting, the presence of white tending to decrease the amount of brindle, to separate the pigmentation into clear-cut areas of black and yellow, and where brindling persists to organize the spots so that black tends to be central and yellow peripheral. The effect of white spotting on tortoise shell has also been discussed by ILJIN (1928). WRIGHT found no change in the ratio of yellow to black areas in relation to increased amounts of white in tricolors of the same stock. In animals of low grade spotting, much brindling is generally still present as well as the segregated colors; in animals of high grade spotting, brindling may occur but is comparatively rare. Frequently in the case of the latter, only one color is present in the spots. These occasional bicolors (black-white, yellow-white) breed, however, as normal tricolors. Whether these effects depend on white spotting as such or on the specific factor *s* is a question which will be considered later.

Of the non-hereditary factors involved in the black-yellow pattern, no statistical analysis has been made but the great irregularity and asymmetry demonstrate clearly their existence.

That sex modified both aspects of the tricolor pattern was shown by WRIGHT (1917, 1920). His studies indicated that males have seven percent more color than females and four percent more black in the colored spots.

Of tortoise shell patterns in other mammals, that of the cat is of particular interest here. The heterozygous condition of the sex-linked black and yellow factors produces a mixture of black and yellow. WHITING (1919) found that the presence of white tended to decrease the amount of brindle and to separate the coat into clear areas of black and yellow. He was unable to discover any increase in the amount of yellow relative to black. Black and yellow spotting in the rabbit (Japanese variety) was reported by CASTLE (1924) and PUNNETT (1924) and is due to an allele in the black series as is the case for the guinea pig. An additional case in mammals is that in swine (WRIGHT 1918). The Berkshires and Poland-Chinas are black with white largely at the extremities. Analysis of crosses indicated that the white of this pattern represented tortoise shell yellow diluted to white. Crosses between Berkshires or Poland-Chinas and red breeds produce well-defined tortoise shells. The data of LUSH (1921) and WARWICK (1926, 1931) indicate that tortoise shell differs from self red by one major factor whereas the extent of black in tortoise shells depends on multiple factors. The black-white extended tortoise shell of Berkshires and Poland-Chinas contrasts with the black-white pattern of Hampshires which resembles somewhat that of the Dutch rabbit and probably belongs in the same category with the spotting patterns of color and white.

Analysis of cross between tricolor and self-yellow

The tricolor stock used was DS (figure 1, CHASE 1939). Its origin from strains D and 2 has been described in Part I. It should be noted that strain D, constituting seven-eighths of its ancestry, had been closely inbred since 1906 (first by PROFESSOR W. E. CASTLE and later by PROFESSOR SEWALL WRIGHT). While considerable fixation might be expected from

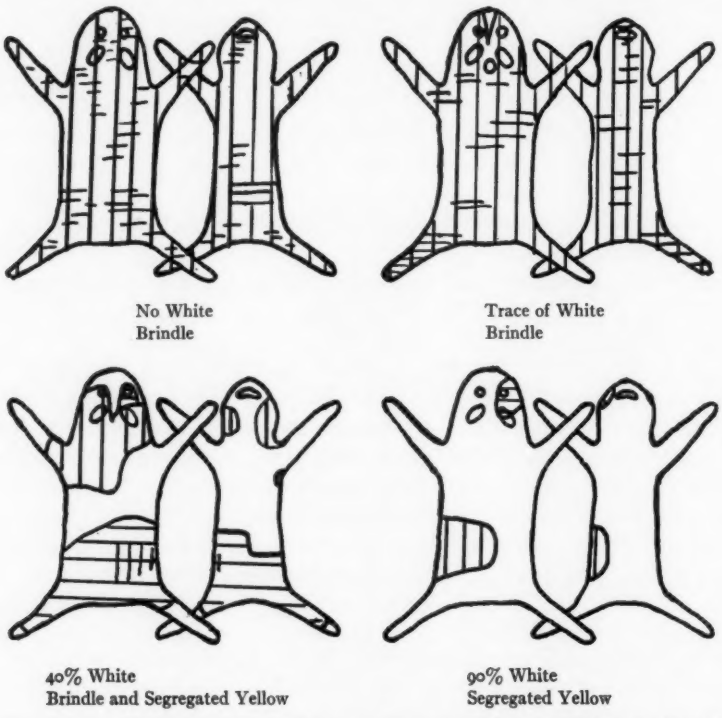

FIGURE 1.—Typical patterns of e^p animals. Areas are represented by vertical lines for black, by horizontal lines for yellow, and by clear regions for white.

the procedure involved in deriving DS, persistent heterozygosity was shown by a parent-offspring correlation of +.182 as compared with the similar correlation of a random bred stock, +.191. The average in five inbred stocks was only +.012 (WRIGHT and CHASE 1936).

Strain 2 has high grade spotting (median percentage white 93.2 in males, 94.6 in females), strain DS has low grade spotting (6.5 in males, 12.0 in females), the difference being due solely to the seven-eighths of its modifiers which came from D. It was desirable to learn something of

the nature of these modifiers and although the lack of homozygosity in DS made it rather unsatisfactory material for this purpose, some data were obtained. Strains DS and 2 were crossed giving 80 animals in the F_1 generation and 72 in the F_2 generation. The ranges were similar (table 1), the slightness of the increase of variability of F_2 over F_1 indicates multiple factors.

TABLE 1

Strain 2 by strain DS. Distribution of 2, DS, F_1, F_2, and backcross to DS. Males, females, and reciprocal crosses combined.

CROSS	GRADE OF WHITE SPOTTING*											NUMBER	MEDIAN PERCENT
	o	x	1	2	3–5	6–8	9–11	12–14	15–17	18–20	W		
2	—	—	—	—	—	3	33	96	343	1125	50	1650	93.90
DS	—	29	37	35	30	10	1	2	1	—	—	145	9.30
F_1	—	—	—	—	12	15	17	18	12	6	—	80	52.50
F_2	—	—	2	2	8	7	17	16	16	4	—	72	57.50
Backcross to DS	—	1	5	4	7	6	3	1	—	—	—	27	21.25

* o—no white, x—trace of white; 1 = 2.5–7.5% white; 2–20 = 5% intervals up to all white (W).

Strain DS (tricolor) was crossed with yellows to obtain data on the dominance of e^p over e and on the relation of tortoise shell and white spotting. A stock of yellows having the dilution factor $c^k c^k$ (WRIGHT 1925) was used. Strain DS of constitution *CC* shows intense yellow (or red). Phenotypically the parental types were black-red-whites (sse^pe^pCC) and yellows (mostly $SSeec^kc^k$). The distribution of intense (*C*) and dilute (c^k) young seems to present nothing of interest and is not considered.

Table 2 gives the total F_1 and F_2 progeny according to sex, color, and the amount of white. The F_1 generation from the usual self yellow parent, consisted of black-reds with no white (61 percent) or a little white (D modifiers frequently repressing the appearance of white in these *Ss* animals). The segregants in the F_2 generation from the matings *inter se* of the F_1 heterozygotes were the animals used in this analysis. The original animals (grandparents) from which all the F_2 descended were three tricolor males, two tricolor females, two yellow males, and seventeen yellow females. Having about 31 pairs of chromosomes (60 to 65 chromosomes, LEAGUE 1928, MOLS 1928), F_2 segregants with respect to any one locus should have, on the average, at least 97 percent of the same modifiers. There is thus little chance that results have been due to linkage to any important extent.

TABLE 2

Distribution of F_1 and F_2 from cross between tricolor and yellow.

MATING	TYPE	AMOUNT OF WHITE IN PERCENT						NUMBER
		0	TRACE	5	10–20	25–50	55–90	
$ssee^p e^p$	$e^p e$ ♂	23	11	1	—	—	—	35
F_1 ×	$e^p e$ ♀	26	19	—	—	—	—	45
SSee								
$ssee^p e^p$	$e^p e$ ♂	2	—	—	—	1	2	5
F_1 ×	$e^p e$ ♀	1	2	—	1	1	2	7
Ssee								
Sse^p*e*	e^p — ♂	71	19	5	12	9	3	119
F_2 ×	e^p — ♀	43	15	3	4	7	3	75
Sse^p*e*	*ee* ♂	17	10	1	5	2	2	37
	ee ♀	19	9	4	5	6	3	46
Sse^p*e*	e^p — ♂	5	—	—	2	—	—	7
F_2 ×	e^p — ♀	7	2	2	—	5	2	18
ssee^p	*ee* ♂	1	1	—	—	—	—	2
	ee ♀	—	1	—	—	1	—	2

For 277 F_2's (those from $Ss \times Ss$) the expectation is 25 percent *SS*, 50 percent *Ss*, and 25 percent *ss*. For the remaining 29 F_2's (from $Ss \times ss$) the expectation is 50 percent *Ss* and 50 percent *ss*. Combining, the expectation for the total F_2 is found to be 22.6 percent *SS*, 50 percent *Ss*, and 27.4 percent *ss*. Assuming that in this cross *SS* is always self and *ss* is always at least 2.5 percent white, the total F_2 can be analyzed as in table 3. In

TABLE 3

Percentage analysis of S, s in F_2.

GENOTYPE	AMOUNT OF WHITE IN PERCENT				TOTALS
	0	TRACE	5	10–90	
SS	22.6	—	—	—	22.6
Ss	30.7	18.6	0.7	—	50.0
ss	—	—	4.2	23.2	27.4
	53.3	18.6	4.9	23.2	100.0

the F_2 generation over half (58 percent) of the self animals were probably heterozygous for the white spotting factor. From another viewpoint, about 61.3 percent of *Ss* in F_2 were self. This agrees well with the 61 percent self of the F_1's that were *Ss*. The total F_2 distribution indicates that the line of cleavage between *Ss* and *ss* is in the category of five percent

(grade 1). The overlap may extend beyond this category (2.5 to 7.5 percent white) but from table 3 as well as from breeding tests and the F_1 distribution there is probably very little overlap. In this experiment males and females agree so closely in the amount of white that for each the line of cleavage falls within this category of five percent (males at the line between trace and five percent). Of the *ss* animals most are in the region from 10 to 50 percent, only one having as much as 90 percent white. With possibly rare exceptions the genotypes and phenotypes are thus: *SS* self; *Ss* self or a trace of white, rarely in the class 2.5 to 7.5 percent white; *ss* rarely in the class 2.5 to 7.5 and ranging up to 90 percent.

TABLE 4

Tests of the F_2 tortoise shells and tricolors.

MATING	PROGENY		
	SEX	e^pe	ee
♀ e^pe^p (18)×♂ ee	♂	43	—
	♀	44	—
♀ ee×♂ e^pe^p (27)	♂	115	—
	♀	119	—
♀ e^pe (40)×♂ ee	♂	25	29
	♀	34	29
♀ ee×♂ e^pe (49)	♂	85	74
	♀	53	75

The results in F_2 (see table 2) from *Ss* by *Ss* are decidedly irregular both with respect to sex ratio (of tortoise shells and tricolors) and with respect to the segregation of yellow (in females). The probability that chance might yield such a series of deviations is only .003 ($\chi^2 = 14.0$, three degrees of freedom). Nevertheless, this probably is merely an extreme chance deviation since on adding the small number of F_2's from *Ss* by *ss*, which happen to show a compensating deviation, χ^2 falls to 7.6 and P rises to .06.

Tortoise shells and tricolors of the F_2 generation have been tested as far as possible to determine which animals are e^pe^p and which, e^pe. The test involves backcrossing to a yellow (*ee*). Of 129 animals having had test progeny at this time, 89 have had at least one yellow offspring and are e^pe, 40 have had no yellows among from two to 24 offspring and are probably e^pe^p (three exceptions probable). Table 4 gives the test data. Of the 40 classified as e^pe^p, three have had two young, five have had three young, six have had four young, and the remainder have had five or more.

A few of these (expectation 3.0) are no doubt $e^{p}e$ but there should be no serious statistical error in treating this group as $e^{r}e^{p}$.

In order to describe in detail the interrelationships between the white and yellow spotting patterns, a precise and objective method of recording the latter was necessary. The amount of white was calculated by dividing the coat into 20 squares on a tracing cloth and reliable estimates were obtained from the recorded drawings (WRIGHT and CHASE 1936). The measurement of tortoise shell was more difficult. The same 30 points were used as in the correlation studies (figure 6, CHASE 1939). These could be detected objectively on each animal. Such points were the tip of the middle toe on the hind foot, the most dorsal point of the scapula in normal resting position, *et cetera*. By using available points of reference, as umbilicus, mammae, elbows, and so on, the 30 points are sufficiently constant from animal to animal. Hairs were plucked with one movement from the selected point with no regard for the colors present. By this means an average of 50 hairs was obtained, spread out, and the first 25 counted. In this manner the numbers of black, yellow, and white hairs were tabulated for each of the 30 points. If the 25 hairs at a point were of one color, that point was termed "segregated." In a few cases all three colors were plucked at one point, in which case the proportion of colored hairs that were yellow was taken as the grade of brindle. This method, involving 750 hairs for each animal, appears to be a reliable and objective means of making comparisons with respect to the complicated tortoise shell pattern.

Dominance in E series

The problem of dominance is of special interest in multiple allelic series. *E* is completely dominant over the lower alleles, e^{p} and *e*. From PROFESSOR SEWALL WRIGHT'S unpublished data (included here with his permission), only one case of incomplete extension of black has been found among approximately 900 *Ee* guinea pigs (from matings of *Ee* by *ee*). This animal (♀ 9942 of experiment EC-92) was black with the right hind leg red (SSC^{r}). It bred like *Ee* in successive matings with two yellow (*ee*) males.

The uniformity of *ee* (no extension of black) is shown by the occurrence among over 3,000 *ee* animals with no e^{p} in the ancestry of only two with black spots. These animals, also from WRIGHT'S unpublished data, were ♂ 9807 of experiment ED-107 and ♀ 7167 of experiment Yd-f-45. Male 9807 was cream ($SSc^{d}c^{r}$) with a small sepia spot on the left shoulder at the mid-dorsal line. Female 7167 was yellow and white ($Ssc^{d}c^{d}$) with a dark sepia spot between the ears.

Now the question arises as to whether e^{p} is completely dominant over *e*. As noted above, the F_2 tortoise shells and tricolors in my experiment have

been tested as far as possible by mating to yellows to determine if they are homozygous or heterozygous for e^p. The amount of segregation of colors and the percentage of yellow hairs in the colored areas vary greatly among individuals similar in genotype and in amount of white. The ranges of the 40 possible homozygotes and the 89 heterozygotes overlap greatly as shown by the percentages of colored hairs that are yellow (table 5 and figure 2).

TABLE 5

Distribution of tortoise shells and tricolors of F_2 according to the percentage of colored hairs that are yellow, the amount of white, and the genotypes.

	TOTAL e^p— (200)					TESTED e^pe^p (40)					TESTED e^pe (89)					UNTESTED e^p— (71)				
PERCENTAGE OF COLORED HAIRS	0	TRACE	5%	10–20%	25–90%	0	TRACE	5%	10–20%	25–90%	0	TRACE	5%	10–20%	25–90%	0	TRACE	5%	10–20%	25–90%
AMOUNT OF WHITE																				
91–100																				
81– 90		1										1								
71– 80	1		1	1	3					1	1			1	1			1		1
61– 70	1	1		1	3						1	1			1				1	2
51– 60	7	3		3	4		1				6			2	2	1	2		1	2
41– 50	11	3	3	3	3	1					6	2	2	2		4	1	1	1	3
31– 40	17	6	3	4	2	2		1			9	6	2	3	1	6			1	1
21– 30	18	9	2	3	5	4			1	1	9	6		1	2	5	3	2	1	2
11– 20	28	5	1		4	9	1	1		3	9	2			1	10	2			
1– 10	30	3		1	1	10	1		1		6	1			1	14	1			
0	2			1	2	1				1					1	1			1	

In analyzing the distributions of table 5, the total distribution will be considered first with no separation according to the amount of white. The calculations are based on the actual individual percentages rather than on the class percentages. Table 6 indicates that there is no real difference between tested and untested but that there is a clearly significant difference between e^pe^p and e^pe. The standard error of the difference is calculated from the standard deviation of the total distribution ($\sigma = 19.0$) in order to avoid any argument in a circle which might come from the use of the separate standard deviations of e^pe^p and e^pe. This gives a conservative test.

It has been previously discussed that the break between *S-* and *ss* falls approximately between a trace and five percent white. Table 6 gives the analysis for the *S-* animals. Here again, basing the standard error of the difference on the standard deviation of the total ($\sigma = 17.3$), the difference between tested and untested is not significant but the difference between

$e^p e^p$ and $e^p e$ is clearly significant. No appreciable modification results from the consideration of selfs alone (no white). The difference between the means of $e^p e^p$ and $e^p e$ is 4.8 times the standard error (16.2 ± 3.4) if the calculation is based on the separate standard deviations of each class. The difference is 4.05 times the standard error (16.2 ± 4.0) if the calculation is based on the extremely conservative test (σ of the total number of

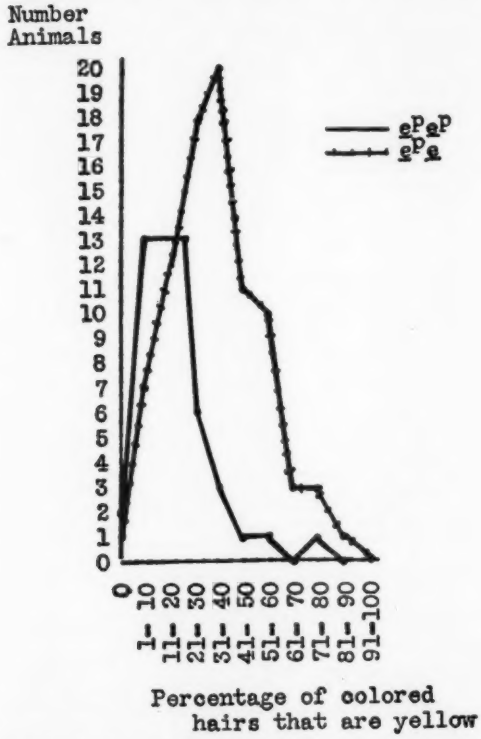

FIGURE 2.—Graphic distribution of the 40 F_2 animals which are tested $e^p e^p$ and of the 89 which are tested $e^p e$.

selfs, 115). Selfs which were *SS* (23) and selfs which were *Ss* (41) indicated no essential difference.

Finally, to complete the analysis, animals with 5 to 90 percent white (presumably nearly all *ss*) were considered (table 6). The difference between $e^p e^p$ and $e^p e$ is as great as in the near-self class but is of less certain significance because of small numbers. There is a considerable difference between tested and untested but it is in the opposite direction from that in the near-self group and therefore probably accidental.

TABLE 6

Percentage of yellow hairs in F_2 tortoise shells and tricolors.

CLASS	NUMBER	MEAN	STANDARD DEVIATION	DIFFERENCE OF MEANS
Tested $S-e^pe^p$	30	16.2		
Tested $S-e^pe$	66	31.9		15.7±3.8
Tested $S-$total	96	27.0		
Untested $S-e^p-$	50	21.1		5.2±3.0
Total $S-e^p-$	146	25.0	17.3	
Tested ss e^pe^p	10	21.3		
Tested ss e^pe	23	38.9		17.6±7.8
Tested ss total	33	33.6		
Untested ss e^p-	21	43.5		9.9±5.8
Total ss e^p-	54	37.4	20.5	
Tested e^pe^p	40	17.5		
Tested e^pe	89	33.7		16.2±3.6
Tested total	129	28.7		
Untested e^p-	71	27.8		0.9±2.8
Total $S-e^p-$	146	25.0		
Total ss e^p-	54	37.4		12.4±3.1
Total e^p-	200	28.4	19.0	

The effect of *ss* on the amount of yellow is rather inconsistent as shown by the great difference in the untested animals and it seems to be less than the effect of heterozygosis in *e*.

The dominance relationships in the black extension series of the guinea pig are then: *E* dominant over e^p and *e*, e^p not completely dominant statistically over *e*. Incomplete dominance would be expected if e^p had a mere quantitative effect, complete dominance would be expected if e^p had a specific effect, one dose of e^p being sufficient to produce this effect.

Relation of tortoise shell and piebald

The black extension series (*E*, e^p, *e*) has been thought not to have any effect on the amount of white. An analysis of the F_2 data, however, suggests that there may be some effect. The genotype S-e^pe has more tendency to have a trace of white than S-e^pe^p; and S-*ee* tends to have more white than S-e^p-. Within tricolors the amounts of white seem not to differ between e^pe^p, e^pe, and *ee*. Before reaching any conclusions concerning the

effect of this series on white, a study involving E as well as e^p and e would be necessary.

White spotting as a modifier of the black-yellow pattern has been mentioned earlier. Tricolors of high grade spotting show an extreme of the segregation of color, frequently even to the point of being bicolors (black-white or red-white). Tabulations were made for two inbred strains, 13E (761 spots) of median 98 percent white, and strain 2 (724 spots) of median 94 percent white. The results presented in table 7 show that most of the spots were completely black or completely red (88 percent in strain 13E, 86.5 percent in strain 2). There were a few spots of brindle. For the less evenly mixed of these, there was a tendency for black to be either at the center as a brindle, at the side on the mid-dorsal line, or at the center as a clear black region. This illustrates the observation first mentioned by WRIGHT (1917) that yellow tends to be peripheral, black, central. One difference between the two strains is evident, the greater tendency for strain 2 to have red spots.

TABLE 7

Color of spots (percentage).

	SEGREGATED COLOR					BRINDLE						
			BLACK AND RED									
STRAIN	BLACK	RED	BOTH AT MID-DORSAL LINE	BLACK AT MID-DORSAL LINE	RED AT MID-DORSAL LINE	EVENLY DISTRIBUTED	RED BRINDLE AT CENTER BLACK AT BORDER	RED SOLID AT CENTER BRINDLE AT BORDER	RED BRINDLE AT MID-DORSAL LINE BLACK LATERALLY	BLACK BRINDLE AT CENTER RED AT BORDER	BLACK SOLID AT CENTER BRINDLE AT BORDER	BLACK BRINDLE AT MID-DORSAL LINE RED LATERALLY
13E	54.1	33.9	1.7	—	—	3.9	0.3	0.5	0.1	1.7	1.4	2.2
2	33.3	53.2	1.9	1.8	—	2.2	0.3	0.1	0.4	1.7	3.6	1.5

A more detailed study of the relation between the two types of spotting has been made on the 200 tortoise shell and tricolor animals of the F_2 generation from the cross of tricolor by yellow considered earlier. Using the hair sample method, 25 hairs at each of 30 points, the animals were tabulated as to solid black, brindle, solid yellow, and solid white at each of these points. In figure 3, the white spotting pattern is indicated. The numbers refer to the actual number of animals having color at the points in question. All except one of the 200 animals had color immediately behind the left eye. Only 145 had color on the tip of the nose, the point least frequently colored. Contours were then made showing levels of frequency. Color is present most frequently in the eye-ear regions and posterior half of the animals, least frequently on the feet, nose, lower lip, throat, chest,

and forehead. A belt across the shoulders shows intermediate frequency. This pattern is similar to the other composite patterns and agrees closely with that of strain DS (figure 1, CHASE 1939), the example of low grade

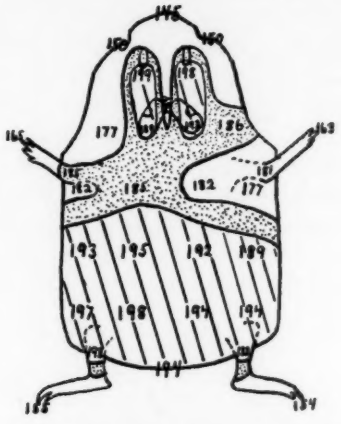

FIGURE 3.—Actual frequency of color at the 30 points. Note: 164 at point between ears.

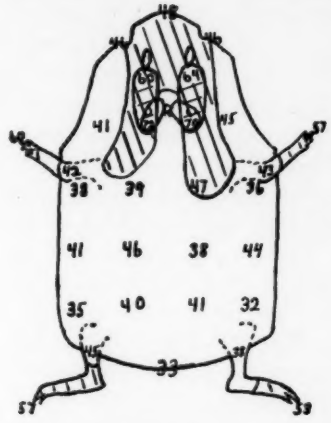

FIGURE 4.—Solid black.

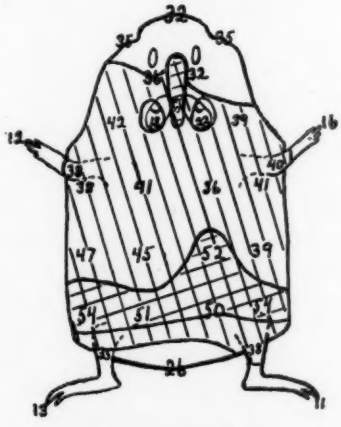

FIGURE 5.—Brindle.

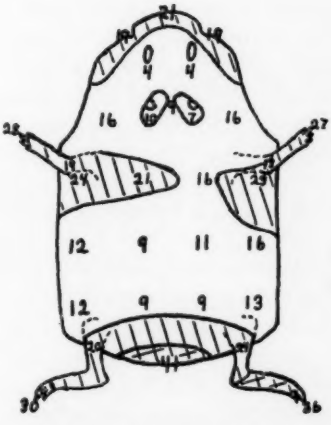

FIGURE 6.—Solid yellow.

FIGURES 4, 5, 6.—Black, brindle, and yellow respectively as percentages of color at the 30 points. Contours separate percentages above and below the average (46 percent for black, 36 percent for brindle, and 18 percent for yellow). A contour at higher frequencies is also shown. Note: for point between ears, 37, 54, and 9 percent respectively.

spotting (making allowance for the fact that F_2 includes *SS*, *Ss*, and *ss*, whereas DS is wholly *ss*).

Figures 4, 5, and 6 show the distribution of segregated black, of brindle, and of segregated yellow in the colored areas. For example, of the 145 cases out of 200 which have color at the tip of the nose, 48 percent are solid black (fig. 4); 32 percent, brindle (fig. 5); and 21 percent, solid yellow (fig. 6). For all points, 46 percent are solid black, 36 percent are mixed, and 18 percent are solid yellow. The nose therefore exceeds the average of the coat as a whole in respect to both segregated colors but is below the average of the coat in respect to brindle. One contour is made at the average percentage, separating in that way those regions of more than average from those of lower than average. Of the former regions those especially high are indicated by an additional contour line. The exceptionally high forehead frequency of the brindle pattern is balanced by lows in black and yellow, the low ear frequencies of brindle are balanced by highs in black, and the low caudal frequency of brindle is balanced by high in yellow. The eye-ear regions are usually black, rarely yellow. Considering the low average percentage of solid yellow, the caudal region is especially high (41 percent). At the extremities, nose and feet, the solid colors occur with higher than average frequency, brindle being especially unusual on the feet. The ventral side, except for the region of the mammae, and the shoulder belt tend to have the remainder of the solid yellow.

A comparison of figures 3 and 6 (nose, feet, and shoulder belt) supports the impression that yellow tends to occupy surface frequently white. Solid black is also frequent on the nose and feet but in addition is most frequent around the ears, regions least frequently white. Brindle is most nearly like the color pattern as a whole (comparison of figures 3 and 5).

Passing from the relationship of white and tortoise shell in locality, there arises the question of the relationship with the amount of white in the animal as a whole. The 200 F_2 animals were tabulated for each point as to solid black, three classes of brindle, one to eight, nine to sixteen, seventeen to twenty-four yellow hairs, and solid yellow. The animals were then divided into five categories each for males and females: no white, a trace of white, five percent white, 10–50 percent white, and 55–90 percent white. In each category the percentages of *colored* points showing solid black, et cetera, were found. The results are presented in figure 7. Selfs have a large amount of black and brindle, a small amount of segregated yellow. Animals with a trace of white show no change in the amount of brindle but a decrease in black and an increase in yellow. At five percent white, a decrease in brindle occurs with both black and yellow increasing. At 10 to 50 percent white (*ss*), the brindle is still decreasing, the black and yellow, increasing. A change other than total amount has appeared for brindle, a relative decrease in the dark brindle (one to eight yellow hairs in the sample of 25). In the last category the numbers are too small to

be relied upon. Sex apparently makes some difference, females having more segregated yellow for a given grade of white than males.

Figure 8 shows the distributions of the tortoise shells and tricolors of F_2 with respect to the percentages of points which are solid black, brindle, and solid yellow. Males and females are combined. The animals are grouped in five classes with respect to white but not all the same classes

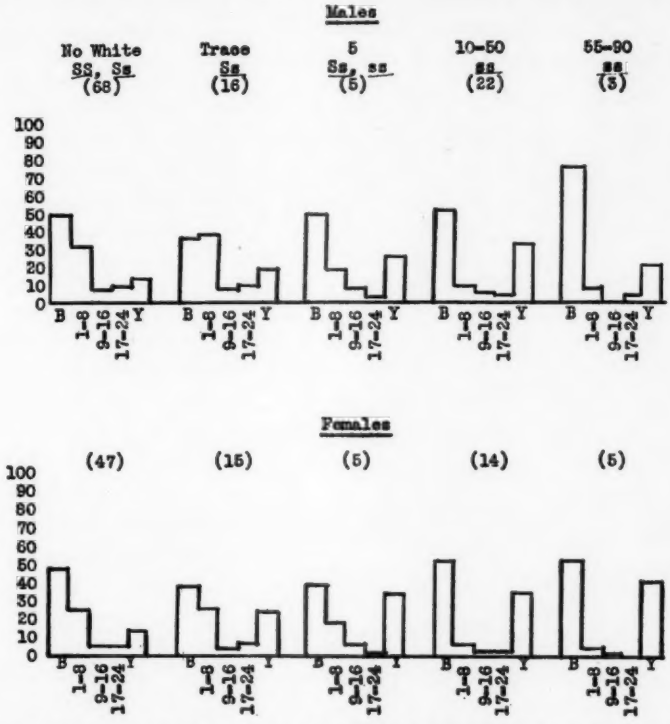

FIGURE 7.—Percentage of *colored* points having all black (B) hairs, 1–8 yellow hairs, 9–16 yellow hairs, 17–24 yellow hairs, and all yellow (Y) hairs in five categories with respect to amount of white. Numbers in parentheses refer to the number of animals (200 in all).

as in figure 7. The figure brings out the great range of individual variability as well as the trend in relation to increasing white. For solid black a decrease occurs with the first appearance of white but subsequently a continued increase. For brindle little difference occurs with the first white but subsequently a continued decrease. For solid yellow a continued increase occurs. A comparison can be made between the frequencies of the colors at specific points (figs. 4, 5, and 6) and the frequencies of the colors at all points in relation to white (figs. 7 and 8). Roughly the comparisons

are: the ears with selfs in which black is frequent, yellow rare; the loin with a trace of white in which brindle is the most frequent; and the "tail," nose, and feet with 10–90 percent white in which brindle is rare, the segregated colors frequent.

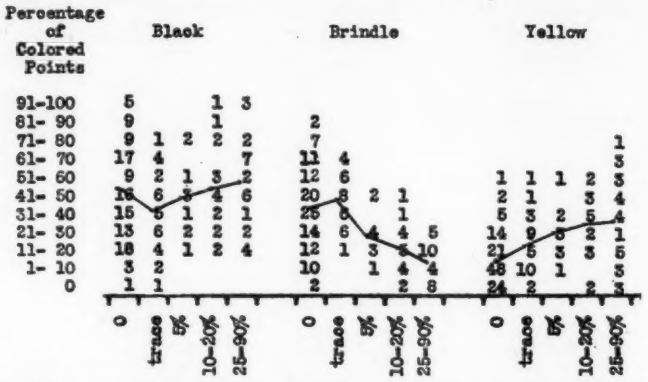

FIGURE 8.—Percentage of colored points that are solid black, brindle, or solid yellow in relation to amounts of white. The 200 animals (males and females) are represented. Mean points found and the trend indicated.

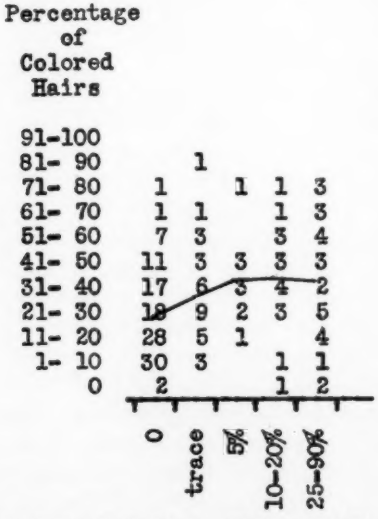

FIGURE 9.—Percentage of colored hairs (750 in selfs) that are yellow in relation to amounts of white. The 200 animals (males and females) are represented. Mean points found and the trend indicated.

Figure 9 shows the percentage of colored hairs (750 in self animals) that are yellow, combining the points. This proportion increases from 23

percent in selfs to 31 percent in animals with a trace of white and to 38 percent in animals with five percent white but increases no further with greater amounts of white. White spotting thus definitely affects both the nature and extent of the yellow spotting pattern.

DISCUSSION

In attempting to interpret gene action in relation to pattern there are a number of alternatives that at once present themselves.

We will consider here both the pattern of color and white on which data has been presented in Part I of this series and the pattern of black and yellow discussed in Part II.

1. Is the pattern of color and white essentially morphological, one of presence or absence of specialized pigment cells; or is it essentially physiological, a pattern of physiological differences in cells similar morphologically?

2. Is the pattern of color and white determined in place, under which there are the alternatives of a local differentiation of morphologically or physiologically distinct cells or a local destruction; or is it a matter of a migration stream which has not reached all parts of the coat? In the latter case the observed pattern might be due in part to local differences in the region of determination and in part to mere distance from the latter.

3. If there is a process of local differentiation either where observed or elsewhere, does it involve nuclear change (somatic mutation) or is it merely cytoplasmic?

4. If there is cytoplasmic differentiation, is it irreversible or is it maintained by local conditions?

Similar questions arise in connection with the pattern of black and yellow.

Embryology

As determined by histologic examination of skin of guinea pig embryos from timed matings (black by black), no pigment was observed up to 41 days 20 hours. Some pigment was found at 42 days 12 hours and at all later ages. Presumably, however, the determination of the pattern was considerably earlier. In Fundulus STOCKARD (1915) showed that chromatophores, at first unpigmented, originated from the wandering mesenchyme cells of the embryo. GOODRICH (1927) describes a similar migratory activity occurring early in another fish, *Oryzias latipes*. For amphibia DUSHANE (1935) has demonstrated the origin of dermal melanophores, not initially pigmented, from the neural crest. Although he was unable to trace epidermal melanophores from that source, he found that they were dependent on neural crest material either for origin or for pigment induction. Although comparable experimental evidence is lacking for mammals, it is possible that here also pigment cells originate from, or are dependent

on, the neural crest and that the color-white pattern is due to an incomplete migration. Observations of the patterns (color-white and black-yellow), the correlation studies, and examples of pigment invasions in transplant experiments suggest a migration stream in general ventrally from the mid-dorsal region and thus in harmony with DUSHANE's observation of a migration of melanophores from the neural crest in amphibia. The pattern would then be due in part to local differences in the region of determination and in part to the extent of migration from the latter.

Transplantation

The results of transplantation experiments bear on some of the questions of gene action in pattern formation. In the guinea pig, skin transplants in the ear have been performed by CARNOT and DEFLANDRE (1896), LOEB (1897), and SAXTON, SCHMECKEBIER, and KELLEY (1936). These were all autoplastic except for some by the first authors. All found spreading of black skin grafted on white and gradual replacement of white skin grafted on black. Hair color, however, was unchanged. SEEVERS and SPENCER (1932) made autoplastic transplants of considerably larger areas (two square centimeters) between regions on the back and belly. In every case the graft continued to produce hair of its original color as long as observed (six to nine months). These grafts included black on red and on white, red on black and on white, and white on black and on red. They found only slight invasion of the skin at the edges of white grafts from the surrounding colored regions. They found complete autonomy of the hair follicles. REED and SANDER (1937) using black-and-tan mice and grafting embryonic tissue on new-born young were able to demonstrate that the color (black or tan) was determined by the seventeenth to the eighteenth day which is before either pigment granules or hair follicles appear.

In fowls, color and pattern were found by DANFORTH and FOSTER (1929) to be fully autonomous (except for sex differences) in transplants between different breeds. WILLIER, RAWLES, and HADORN (1937) transplanted skin ectoderm reciprocally between chick embryos of colored and dominant white breeds at about 75 hours incubation. At 15 days colored down was found on the implants from the colored breed but colored down was also found on the implants from the white breed. The authors note that the latter result might be due either to diffusion of chemical substances or to invasion from the colored host. EASTLICK (1939) has transplanted limb buds in chicks at 40 hours incubation and the evidence suggests a migration of dorsally located cells, possibly from the neural crest, to bring about pigmentation of the limb.

In all cases there was autonomy of cells with the capacity to produce color. Skin and follicles lacking this capacity were also autonomous at

least in the case of white spotting of the guinea pig. This conclusion is of course not invalidated by observations of gradual changes in color if these are correctly interpreted as due either to displacement by the surrounding pigmented skin or to invasion by pigment cells from the latter. The possibility suggested above that the spotting pattern is maintained by local physiological conditions may be considered to be definitely eliminated.

Dopa reaction

The reaction of melanophores to dioxyphenylalanin (dopa), discovered by BLOCH (1917), has been used to demonstrate their presence. SCHULTZ (1925) working with the rabbit and KRÖNING (1930) and RUSSELL (1937, 1937a) working with the guinea pig demonstrated the existence of numerous dendritic cells, blackened by treatment with dopa, in the yellow varieties and in the yellow parts of the tortoise shell pattern of the guinea pig. It seems clear that the tortoise shell pattern depends on physiological differentiation with respect to the production of chromogen rather than on a morphological pattern of presence and absence of pigment cells. GOODRICH (1933) has found a similar situation in the fish *Oryzias latipes*. The dopa reaction is completely negative for the white areas in white spotted animals (in the rabbit, SCHULTZ 1925; in the guinea pig, KRÖNING 1930, RUSSELL 1937, 1937a). There is an indication here that the white spotting pattern (in contrast with the yellow spotting pattern) is determined by actual absence rather than mere physiological alteration of the dendritic cells.

Somatic mutation

In a study of the tricolor pattern in the guinea pig, it is appropriate to consider another class of mosaics, those which are not regularly transmissible as mosaics. Twenty-three cases have been reported for rodents. Eighteen involved heterozygotes in which part of the coat exhibited a recessive condition. Briefly the cases were as follows: one guinea pig, CASTLE (1912); one rat, CASTLE (1922); three guinea pigs, WRIGHT and EATON (1926); seven rabbits, CASTLE (1929); three mice, PINCUS (1929); one mouse, FISHER (1930); two mice, FELDMAN (1935). In one case the animal was homozygous for the color factor (*AA*) agouti guinea pig, but showed a black spot (WRIGHT and EATON 1926). This may have been due to mutations of both *A* genes or possibly to a mutation to a dominant black. The same authors described two quadricolored guinea pigs, black-sepia-red-white. These were homozygous in the intensity factor (*CC*) but the sepia may have been due to an effect of the tortoise shell factor (e^p) since this factor is known to reduce the intensity of black in brindled areas. Apparently these sepia spots were due to such a dilution not associated with the usual mixture of red hairs. The remaining two of

the twenty-three cases of mosaicism are of particular interest because the germ cells were affected. In a dilute-intense mosaic guinea pig, supposedly $c^d c^d$ from its ancestry, a mutation to *C* must have occurred in a cell ancestral to part of the gonad, as indicated by breeding tests, and to two separate intensely colored areas of the skin (WRIGHT and EATON 1926). In a mouse of constitution Cc^r (black) there apparently occurred a mutation of *C* to a new allele, affecting about one-fifth of the gonad and several regions of the skin (DUNN 1934).

In the two germinal-somatic mosaics, in three mosaic guinea pigs, and in one mosaic rat, there was found more than one patch of mutant skin. Assuming that these patches were of common origin, the determination must have been early in development with subsequent separations. The possibility of migratory cells suggests itself.

Regular inheritance of mosaicism as a character has not been indicated in any of these cases but the seven black-maltese mosaic rabbits described by CASTLE (1929) occurred in three successive generations indicating some sort of unstable gene condition.

The possibility that the yellow spotting pattern of the guinea pig, very irregular and at times appearing as single yellow hairs among hundreds of black hairs, is due to the instability of the gene e^p during a certain stage in the development of somatic tissue must be considered. Against this view is the fact that in many generations of brother-sister mating, in several different strains, no evidence has been found that e^p has ever mutated to *E* or *e* in the germ line (WRIGHT and EATON 1926). The possibility of stability in the germ line, instability in the soma, is however indicated by DEMEREC'S (1929, 1929a) results with the gene miniature-gamma in *Drosophila virilis* which in the presence of certain modifiers is almost fully stable in the germ line but mutable in the wings causing mosaics in practically all cases. No conclusive evidence, however, has been obtained in mammals comparable to that in Drosophila.

A relationship between mosaicism and white spotting has been pointed out by DUNN (1934) and FELDMAN (1935). Twenty of the twenty-three rodent cases cited above were white spotted and in most cases at least the mutant tissue occupied regions frequently white. There is a certain similarity here to the influence of white spotting on black and yellow in the guinea pig noted earlier. Another probably related observation is that of PUNNETT (1924) that the yellow in Japanese rabbits (black-yellow) tends to occur in a pattern simulating that of the black-white Dutch rabbits.

Destruction

Ordinary goldfish (*Carassius auratus*) of genotype *TT* acquire their final coloration by a destruction of the melanophores which until about

three months of age give in combination with the xanthophores, which are not usually destroyed, a brown appearance (BERNDT 1925, FUKUI 1927). In the colorless "shubunkins" ($T'T'$) there is an earlier loss of melanophores and a loss of xanthophores as well. In the heterozygote (TT'), known as the common shubunkin, a permanent variegated pattern results from irregular destruction of melanophores and xanthophores (GOODRICH and HANSEN 1931, GOODRICH and NICHOLS 1933). Pattern as a consequence of a destructive process is interesting, but can probably be ignored as a possibility for the tricolor guinea pig in which no progressive effects have been observed.

Realizing that the relation of gene action to pattern determination cannot yet be answered, this series has been concerned with the more precise descriptions necessary for a clearer formulation of the problem. As demonstrated by the composite patterns of white spotting, hereditary modifiers tend to affect the animal as a whole. Non-hereditary modifiers act only locally (correlation studies on inbred strains). Although variability is great for the pattern of black and yellow, evidence is presented that in the multiple allelic series, E, e^p, e, the e^p factor is not completely dominant statistically over e. This suggests a quantitative effect of this intermediate allele. The effect of white on the tortoise shell pattern has been described precisely by the method of hair samples. Segregation of colors increases with the amount of white and the number of yellow hairs increases up to a certain point. That the effect is due to the amount of white rather than directly to the presence of s seems probable since SS and Ss among the selfs apparently do not differ and animals with a trace of white (Ss) do exhibit a noticeable change. The view that solid yellow areas tend to be peripheral and tend to occupy regions frequently white has been corroborated. This appears to be related to the observation that mutant tissue in mutational mosaics also tends to occupy white regions and to occur in white spotted animals (DUNN 1934, FELDMAN 1935).

SUMMARY

A cross between tricolors and yellows is analyzed.

The multiple allelic series for extension of black, E, e^p, e, is considered in respect to dominance. The tortoise shell factor, e^p, is found to be statistically not completely dominant over e. Both e^pe^p and e^pe are highly variable and overlap widely.

A method of using hair samples (750 hairs from each animal) for recording the black-yellow pattern is described. By means of this method an analysis of the relation of tortoise shell and piebald is made. Segregated black and yellow tend to occur on the nose and feet and yellow in particular tends to occupy those localities most frequently white. The segregation

of black and yellow and the relative increase of yellow with the introduction of white are measured. The effects upon the black-yellow pattern vary apparently according to the amount of white, not according to the constitution of the animal with respect to the major pair of alleles, *S*, *s*, alone.

Questions are presented which must be solved in any final interpretation of pattern in relation to gene action. Evidence is considered from studies in various fields, embryology, transplantation, dopa reaction, and somatic mutation.

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